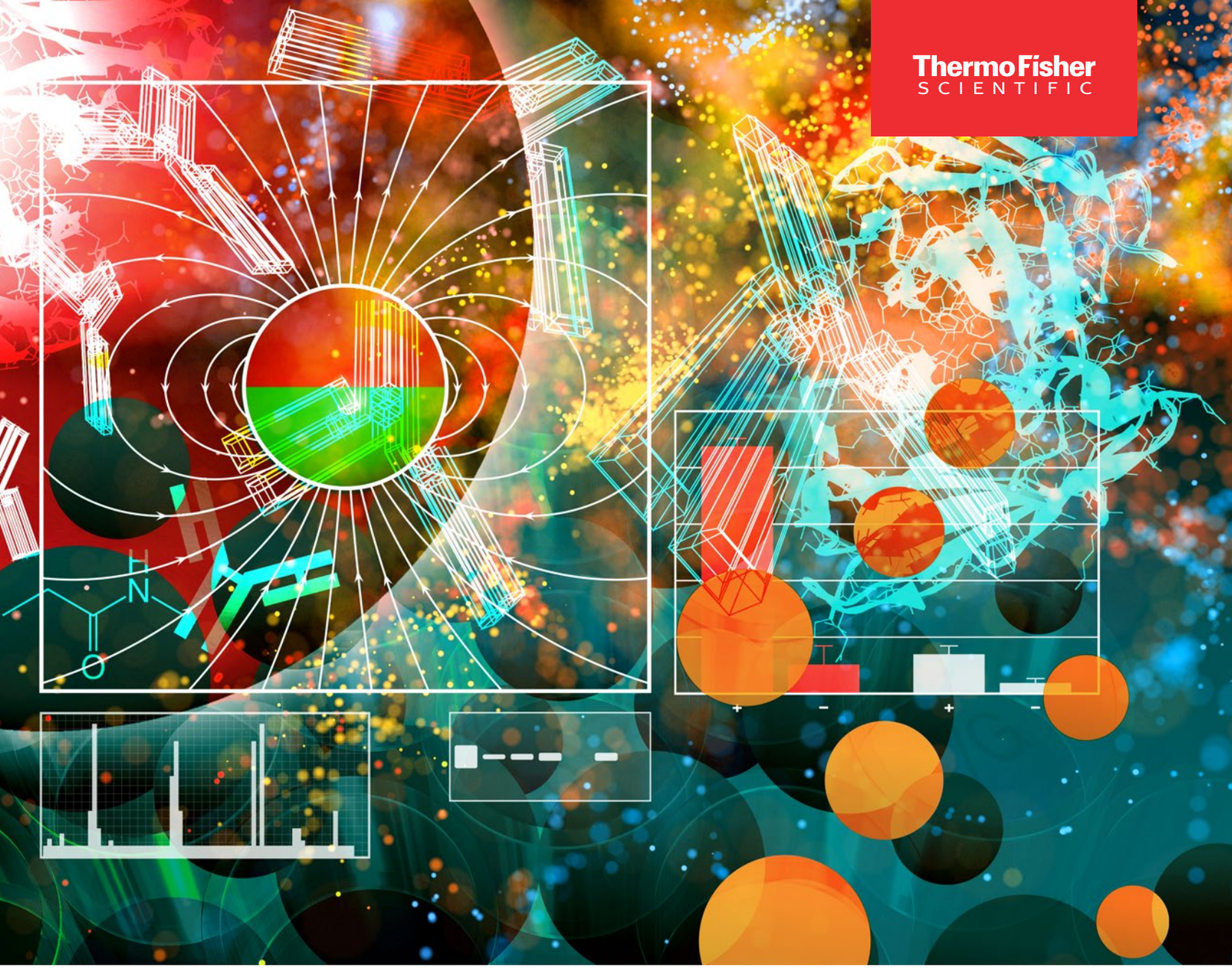


Magnetic bead technology

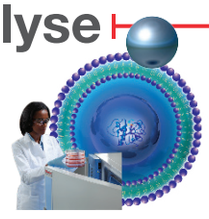
for excellent assay development

thermo scientific

Exceptional bead technology for protein biology

Thermo Scientific™ Magnetic Beads are available for immunoprecipitation and affinity purification as well as custom magnetic particle creation. The high-performance, iron oxide, superparamagnetic particles are validated and optimized for use with high-throughput magnetic platforms, such as the Thermo Scientific™ KingFisher™ Duo, Flex and Apex Instruments. Samples can be analyzed by Western blotting or on Thermo Scientific™ Mass Spectrometers for quantitation of low abundant targets.

lyse



Prepare cells, tissue or fluid samples with Thermo Scientific™ Lysis Reagents

target

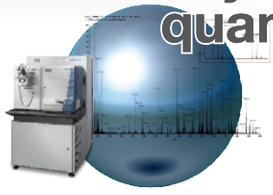
Isolate your target with one of our magnetic beads:

- **Activated magnetic beads** for creating custom affinity resins (pg. 4)
- **Magnetic Protein A/G/L** for immunoprecipitation (pg. 6)
- **Validated kits** for immunoprecipitation, ChIP or RNA-pull downs (pg. 18)
- **Resins** for protein enrichment (pg. 20)

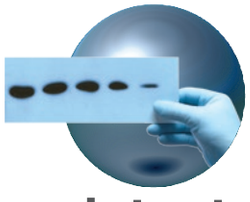
isolate

Choose to run a few samples on the bench or create a high-throughput assay with KingFisher Instruments

identify and quantitate



Identify and quantitate your analyte using Thermo Scientific Mass Spectrometers and labeling reagents



detect

Use our highly sensitive substrates for Western blot detection

Contents

Magnetic protein immobilization beads

Immobilize your ligand for custom magnetic assays

Magnetic Protein Immobilization Beads

Thermo Scientific™ Pierce™ NHS-Activated Magnetic Beads enable covalent, amine-based conjugation of proteins to magnetic beads in a simple mix-and-go format for use in custom affinity purification experiments. The activated magnetic beads contain N-hydroxysuccinimide (NHS) functional groups that react with primary amines, forming stable amide linkages. Once they are covalently attached, the immobilized proteins are highly resistant to leaching from the bead surface. When prepared beads are used in experiments, nonspecific binding is negligible because nonreacted NHS-ester groups are thoroughly blocked during the coupling procedure

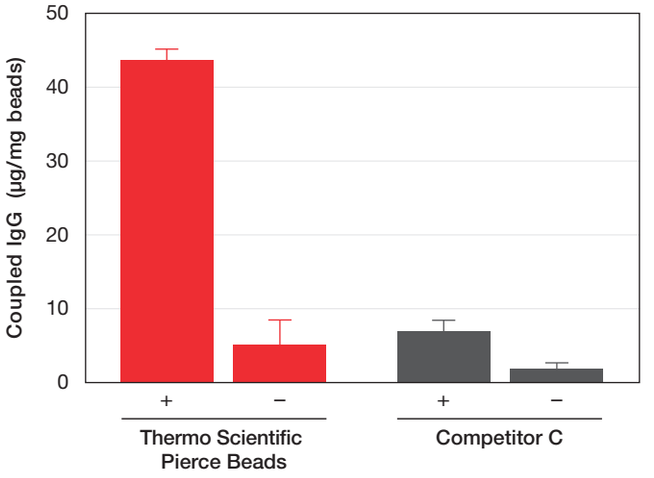


Figure 1. Significantly better coupling capacity with Thermo Scientific Pierce NHS-Activated Magnetic Beads. Rabbit IgG (1 mg/mL) was coupled in PBS for two hours at pH 7.2 to 3 mg each of Pierce NHS-Activated Magnetic Beads and NHS crosslinked, beaded-form of agarose magnetic beads (competitor C). Negative control beads (–) were prepared by quenching or blocking using respective manufacturer protocols. Bound protein was measured using the Thermo Scientific Pierce 660 nm Protein Assay by subtracting the amount of protein in the flow-through from the amount loaded. The Pierce NHS-Activated Magnetic Beads coupled more than four times as much protein as the equivalent amount of competitor C NHS crosslinked, beaded-form of agarose magnetic beads.

Highlights

- **High capacity**—at least four times greater binding capacity than NHS-activated magnetic beads from other suppliers
- **Easy to use**—immobilize in a simple one-step reaction with minimal hands-on time
- **Safe**—no hazardous chemicals (e.g., sodium cyanoborohydride and cyanogen bromide) needed
- **Ligand compatible**—use with nearly any primary amine-containing compound or affinity ligand to immobilize
- **Low nonspecific binding**—the bead surface is pre-blocked and any nonreacted NHS-ester groups are fully quenched
- **Protocol compatible**—protein coupling to the beads and downstream applications can be performed manually or by automation (e.g., KingFisher Instruments)

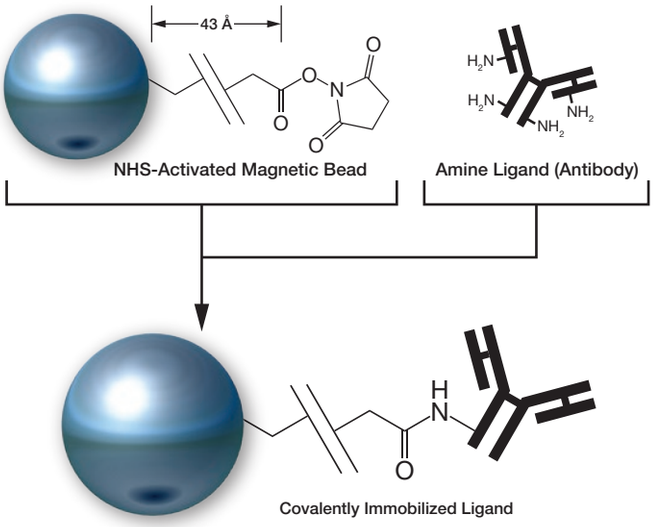


Figure 2. Reaction scheme for conjugation of protein onto Thermo Scientific Pierce NHS-Activated Magnetic Beads.

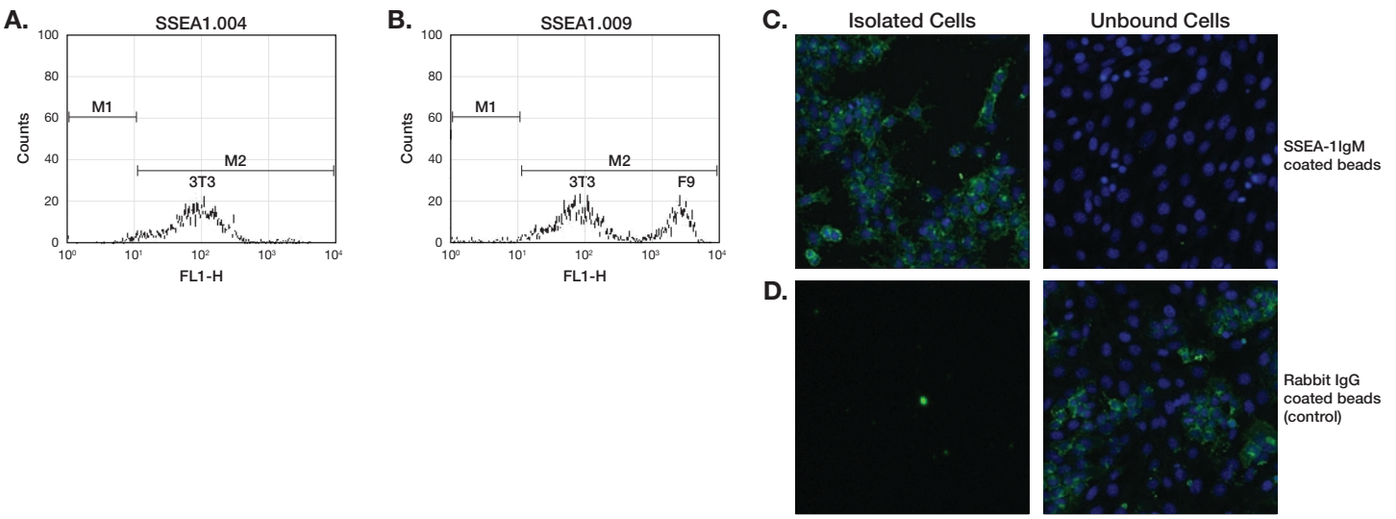
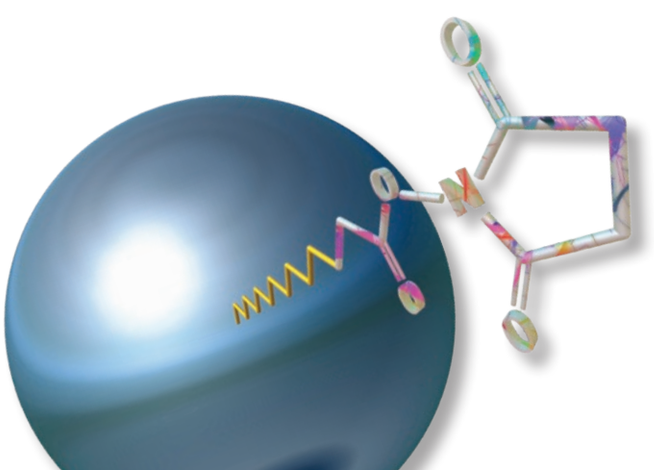


Figure 3. Effective cell separation. Pierce NHS-Activated Beads were coated with Thermo Scientific Stage-specific Embryonic Antigen 1 (SSEA-1) mouse IgM or rabbit IgG as a negative control. The beads were incubated with a 50:50 co-culture of F9 mouse embryonal carcinoma cells (SSEA-1 positive) and NIH 3T3 cells (SSEA-1 negative) for 20 minutes at 4°C. The beads were collected on a magnetic stand and the unbound cell fraction was evaluated by flow cytometry using anti-SSEA-1 mouse IgM and goat anti-mouse IgM-fluorescein. **Panel A** shows F9 cells were selectively depleted with anti-SSEA-1-coated NHS- activated magnetic beads. **Panel B** shows neither cell type bound to the negative control (rabbit IgG-coated NHS magnetic beads). Both the bead-bound and unbound cell fractions were cultured for 24 hours, fixed and then stained with mouse anti-SSEA-1 antibody, Thermo Scientific DyLight 488 conjugated goat anti-mouse IgM and Hoechst nuclear stain. Cells were visualized using the Thermo Scientific ToxInsight Platform. **Panel C** shows that the SSEA-1 antibody coated magnetic NHS beads effectively separated the F9 cells from the NIH 3T3 cells. As expected, the rabbit-IgG-coated magnetic NHS beads (Panel D) did not bind either cell type, and the corresponding unbound fraction contained a 50:50 ratio of both cell types.

Table 1. Properties of Thermo Scientific Pierce NHS-Activated Magnetic Beads.

Composition	*N*-hydroxysuccinimide (NHS) functional groups on a blocked magnetic bead surface
Magnetization	Superparamagnetic (no magnetic memory)
Mean Diameter	1 µm (nominal)
Density	2.0 g/cm^3
Bead Concentration	10 mg/mL in DMAC
Binding Capacity	≥26 µg of rabbit IgG/mg of beads



Ordering information

Description	Quantity*	Cat. No
Pierce NHS-Activated Magnetic Beads Sufficient for: Binding ≥ 26 µg of rabbit IgG/mg of beads	1 mL	88826
Pierce NHS-Activated Magnetic Beads Sufficient for: Binding ≥ 26 µg of rabbit IgG/mg of beads	5 mL	88827

* Available in bulk quantities

Magnetic immunoprecipitation beads

Build your own immunoprecipitation assay

The Thermo Scientific™ Pierce™ Protein A/G Magnetic Bead is a single particle that is compatible with all commonly used antibodies for immunoprecipitation (IP). These beads are coated with genetically engineered Protein A/G, a recombinant protein that combines the IgG binding domains of both Protein A and Protein G. This combination enables the capture of antibodies from a wider range of species and isotypes than either protein alone. Using our crosslinker chemistry, you can immobilize an antibody onto the magnetic particle and prevent IgG contamination in your immunoprecipitated sample.

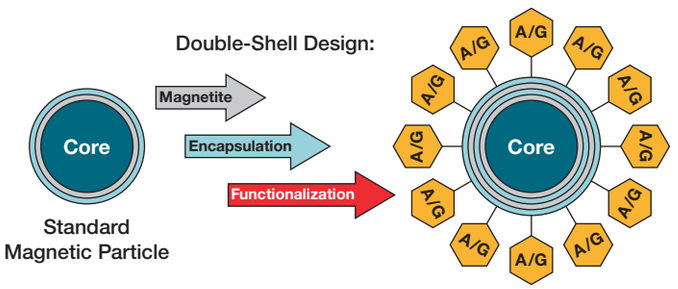


Figure 4. Diagram of Thermo Scientific Pierce Protein A/G Magnetic Beads. The magnetic particles are 1 µm in diameter and are specially manufactured with two layers of magnetite and encapsulation. Recombinant Protein A/G is coupled to the bead surface.

Table 2. Properties of Thermo Scientific Pierce Magnetic Protein A/G Beads.

Composition	Magnetite-coated polymeric beads blocked and covalently coated with a monolayer of recombinant Protein A/G
Mean Diameter	1 µm (nominal)
Density	2.0 g/cm^3
Bead Concentration	10 mg/mL in water with sodium azide
Binding Capacity	55 to 85 µg of rabbit IgG/mg magnetic beads

Highlights

- **Compatible**—one magnetic bead type that can capture most primary antibodies
- **Fast**—immunoprecipitating in as few as 30 minutes helps reduce nonspecific binding and improves the capture of transient protein complexes
- **Clean**—immobilize your antibody to prevent contamination in your eluate
- **Resistant**—no leaching of Protein A/G in the presence of detergents, low pH buffers or common mass spectrometry solvents
- **Efficient**—immunoprecipitate with half the recommended volume of magnetic particles compared to other magnetic beads

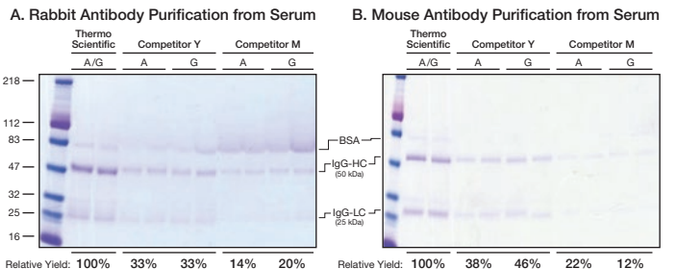


Figure 5. Thermo Scientific Pierce Protein A/G Magnetic Beads isolate significantly more IgG from rabbit and mouse serum with less background than other brands of Protein A and Protein G magnetic particles. Using a KingFisher Flex Instrument with a 96 deep well plate, IgG was purified from 5 mg of rabbit and mouse serum using 50 µL of Pierce Protein A/G Magnetic Beads, Protein A or G magnetic beads from competitor Y or M. The beads were washed with Tris-buffered saline containing 0.05% Tween-20 (TBST), incubated for one hour with serum diluted in TBST, washed three times, and then eluted with 0.1 M glycine, pH 2.8 for 10 minutes at room temperature. The eluates were resolved by SDS-PAGE and stained with Thermo Scientific Imperial Protein Stain. **Panel A:** Rabbit serum; **Panel B:** Mouse serum. The IgG heavy chain bands were quantified by densitometry. The values for each set of duplicate bands were averaged and expressed as a percentage of the average for the Pierce Protein A/G Magnetic Beads.

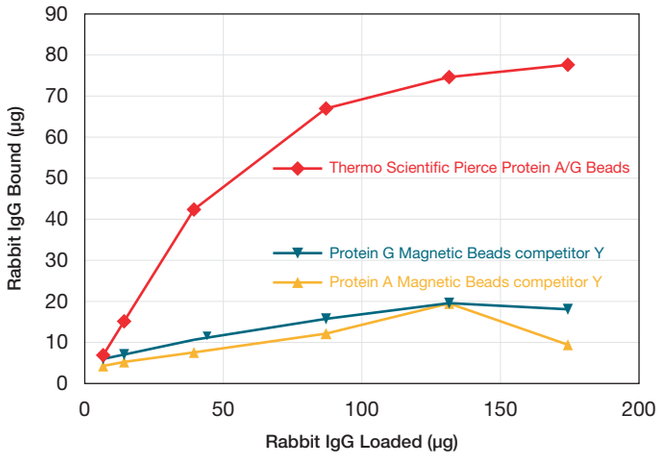


Figure 6. The rabbit IgG binding capacity of Thermo Scientific Pierce Protein A/G Magnetic Beads is approximately four times greater than that of other Protein A and Protein G beads. Pierce Protein A/G Magnetic Beads or Protein A or Protein G magnetic beads from competitor Y were added to a 96 deep-well plate (1 mg beads per well). Using the Thermo Scientific KingFisher 96 Instrument, the beads were incubated for one hour with varying amounts of purified rabbit IgG (20 to 200 µg). After binding, the samples were eluted at 96°C with SDS-PAGE reducing sample buffer. Binding was calculated using the Thermo Scientific Pierce BCA Protein Assay.

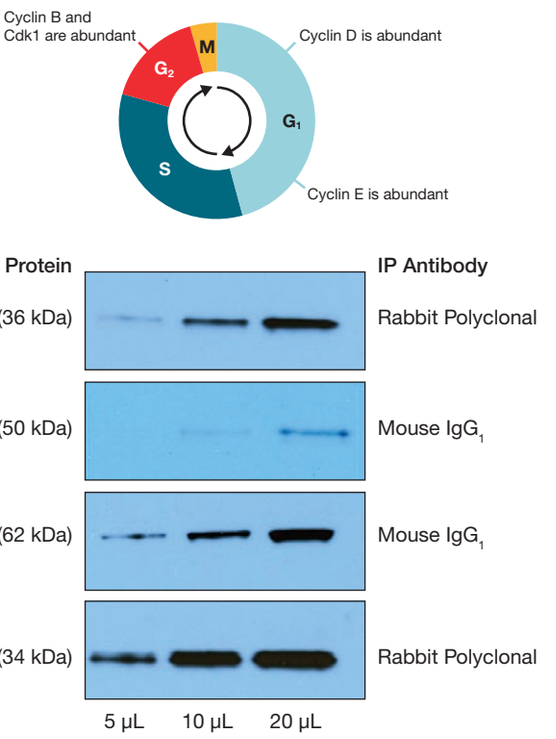
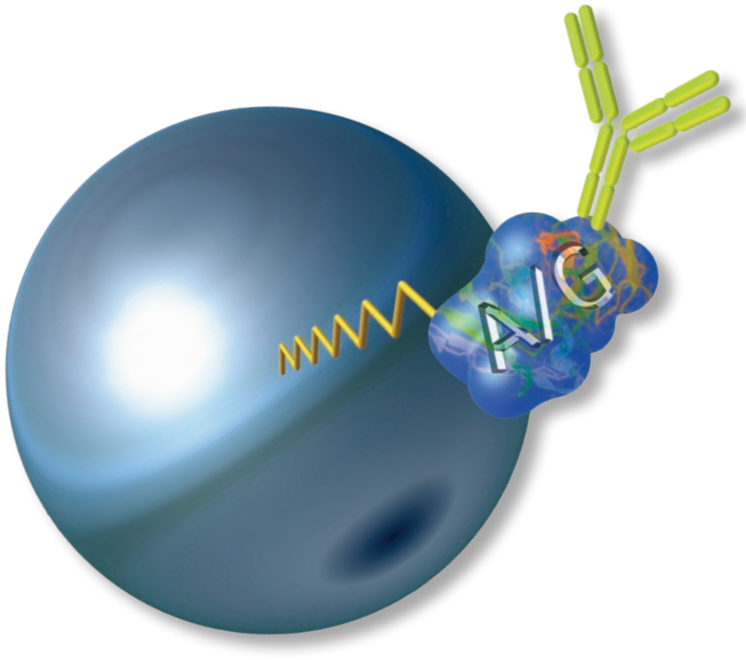


Figure 7. The Thermo Scientific Pierce Protein A/G Magnetic Beads effectively immunoprecipitate cell cycle proteins Cyclin D, Cyclin E, Cyclin B and Cdk1. U2OS (human osteosarcoma) cells were synchronized at G0 followed by growth in 20% fetal bovine serum for 4, 6 and 18 hours before harvest. The cells were lysed in IP lysis/wash buffer, and 0.75 mg of lysate (per sample) was incubated with anti-Cyclin D (rabbit polyclonal), anti-Cyclin E (mouse IgG1), anti-Cyclin B (mouse IgG1) or anti-Cdk1 (rabbit polyclonal) antibodies overnight at 4°C. The Pierce Protein A/G Magnetic Beads were added (50 µL each) to a 96 deep-well plate and immunoprecipitations were performed using the KingFisher Flex Instrument. Eluted sample volumes of 5 µL, 10 µL and 20 µL were resolved by SDS-PAGE and analyzed by Western blot.



Ordering information

Description	Quantity*	Cat. No
Pierce Protein A/G Magnetic Beads Sufficient for: Binding 55 to 85 µg rabbit IgG/mg beads.	1 mL	88802
Pierce Protein A/G Magnetic Beads Sufficient for: Binding 55 to 85 µg rabbit IgG/mg beads.	5 mL	88803

* Available in bulk quantities

Magnetic immunoprecipitation beads, cont.

Build your own immunoprecipitation assay

Thermo Scientific™ Pierce™ Protein A/G Magnetic Agarose Beads provide a fast, convenient method for purification of immunoglobulins from serum, cell culture supernatant, or ascites. For antibody purification, the beads are incubated with the antibody sample and then magnetically separated from the supernatant. Nonspecifically bound serum or host cell protein can be washed away before dissociating bound antibody with elution buffer. The beads are removed from the solution manually using a magnetic stand or by automation using an instrument such as the Thermo Scientific™ KingFisher™ Flex Magnetic Particle Processor. Automated instruments are especially useful for higher throughput purification and screening of purification conditions.

Pierce Protein A/G Magnetic Agarose Beads contain a 50.5 kDa Protein A/G recombinant fusion protein that is covalently attached to a magnetite-embedded agarose core particle. These beads are not simply a mixed immobilization of separate Protein A and Protein G polypeptides, nor are they a mixture of Protein A magnetic beads and Protein G magnetic beads. The recombinant chimeric Protein A/G combines four IgG binding domains from Protein A and two binding domains from Protein G, making it a more general and convenient tool for purifying immunoglobulins.

Highlights

- **Engineered**—immobilized recombinant fusion protein of the antibody-binding domains of Protein A and Protein G enables IgG purification from nearly any mammalian species
- **High capacity**—sufficient for both routine and demanding purification procedures
- **Low non-specific binding**—optimized purification protocol results in better IgG purification
- **Flexible**—convenience of IgG binding domains of both Protein A and Protein G on one bead
- **Compatible**—beads are compatible with manual and automated applications
- **Inert and stable**—superior manufacturing method immobilizes Protein A/G by leach-resistant covalent bonds

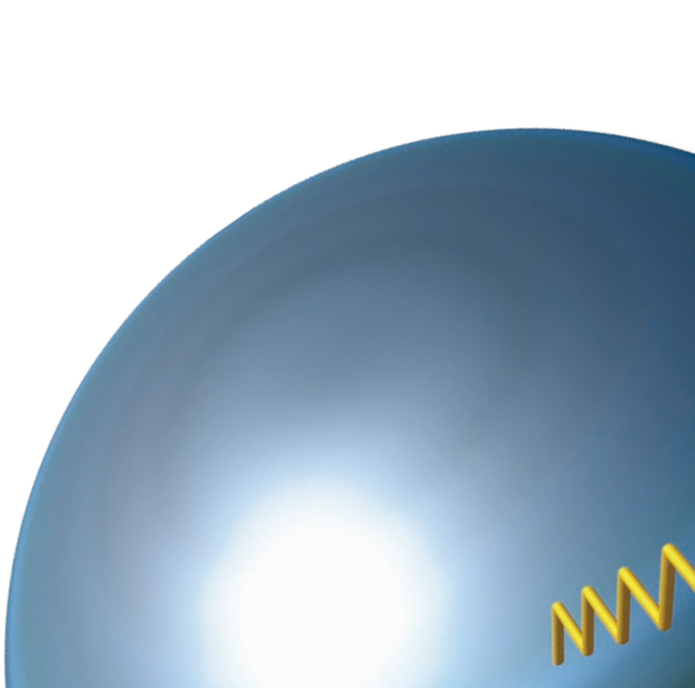
The high-performance, magnetite-containing, superparamagnetic magnetic agarose beads are validated and optimized for use with high-throughput magnetic platforms, such as the KingFisher 96 and KingFisher Flex magnetic particle processors, but the beads also enable premium performance for simple benchtop purification applications using an appropriate magnetic stand.

Our recombinant Protein A/G binds to all human IgG subclasses, making it the ideal choice for purification of polyclonal or monoclonal IgG antibodies whose subclass identities have not been determined. In addition, it binds to IgA, IgE, IgM and (to a lesser extent) IgD. Protein A/G binds well to all mouse IgG subclasses but does not bind mouse IgA, IgM, or serum albumin. This makes Protein A/G an excellent tool for purification and detection of mouse monoclonal antibodies from IgG subclasses, without interference from IgA, IgM, and murine serum albumin. Individual subclasses of mouse monoclonals are likely to have a stronger affinity to the chimeric Protein A/G than to either Protein A or Protein G.

Product characteristics

Table 3. Characteristics of the Thermo Scientific™ Pierce™ Protein A/G Magnetic Agarose Beads.

Composition	Recombinant Protein A/G covalently attached to magnetic, highly crosslinked agarose supports
Magnetization	Ferrimagnetic with low remanence
Mean Diameter	10–40 µm
Bead concentration	25% slurry in phosphate buffered saline, 0.01% Tween™-20 Detergent, 0.05% sodium azide
Binding capacity	>40 mg rabbit IgG/mL settled beads



Magnetic vs. non-magnetic agarose purification

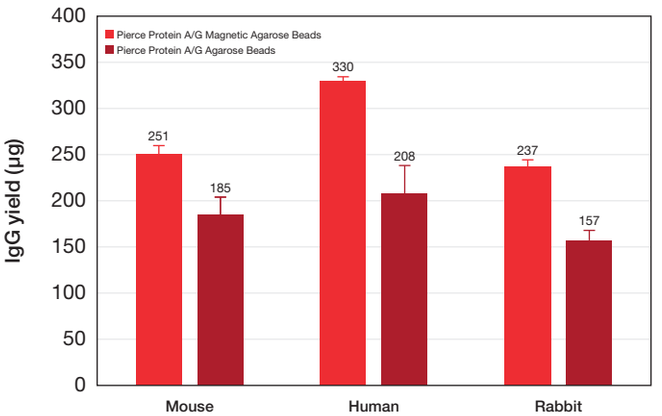


Figure 8. Thermo Scientific™ Pierce™ Protein A/G Magnetic Agarose Beads provide higher purification yields in comparison to non-magnetic agarose. Serum (50 µL) was diluted 10X with binding buffer and added to washed Pierce Protein A/G Magnetic Agarose Beads or Pierce Protein A/G Agarose Beads (10 µL). IgG was purified following the manufacturer's protocols. IgG yield was estimated by absorbance of IgG at 280 nm. All purifications were done in duplicate.

IgG isotype binding

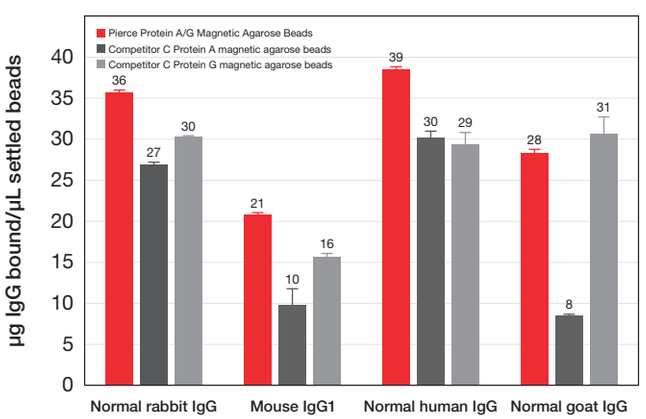
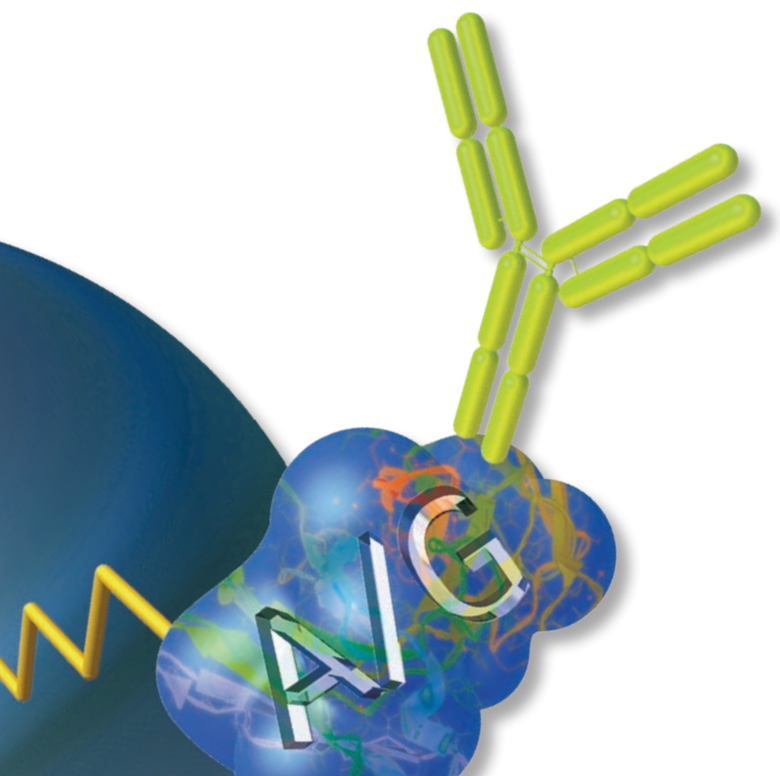


Figure 9. Thermo Scientific™ Pierce™ Protein A/G Magnetic Agarose Beads provide high binding capacity to Rabbit IgG, Mouse IgG1, Human IgG, and Goat IgG. Different IgG isotypes (0.5 mg) were added to 10 µL settled beads (Pierce Protein A/G Magnetic Agarose Beads, competitor C Protein A magnetic agarose beads, or Competitor C Protein G magnetic agarose beads) and allowed to bind for 1 hour. The magnetic beads were then captured and the amount of IgG in the flow-through was calculated by A280. Bound IgG was calculated as total IgG in flow-through subtracted from IgG loaded (n = 3).



Magnetic immunoprecipitation beads, cont.

Build your own immunoprecipitation assay

IgG purification yield

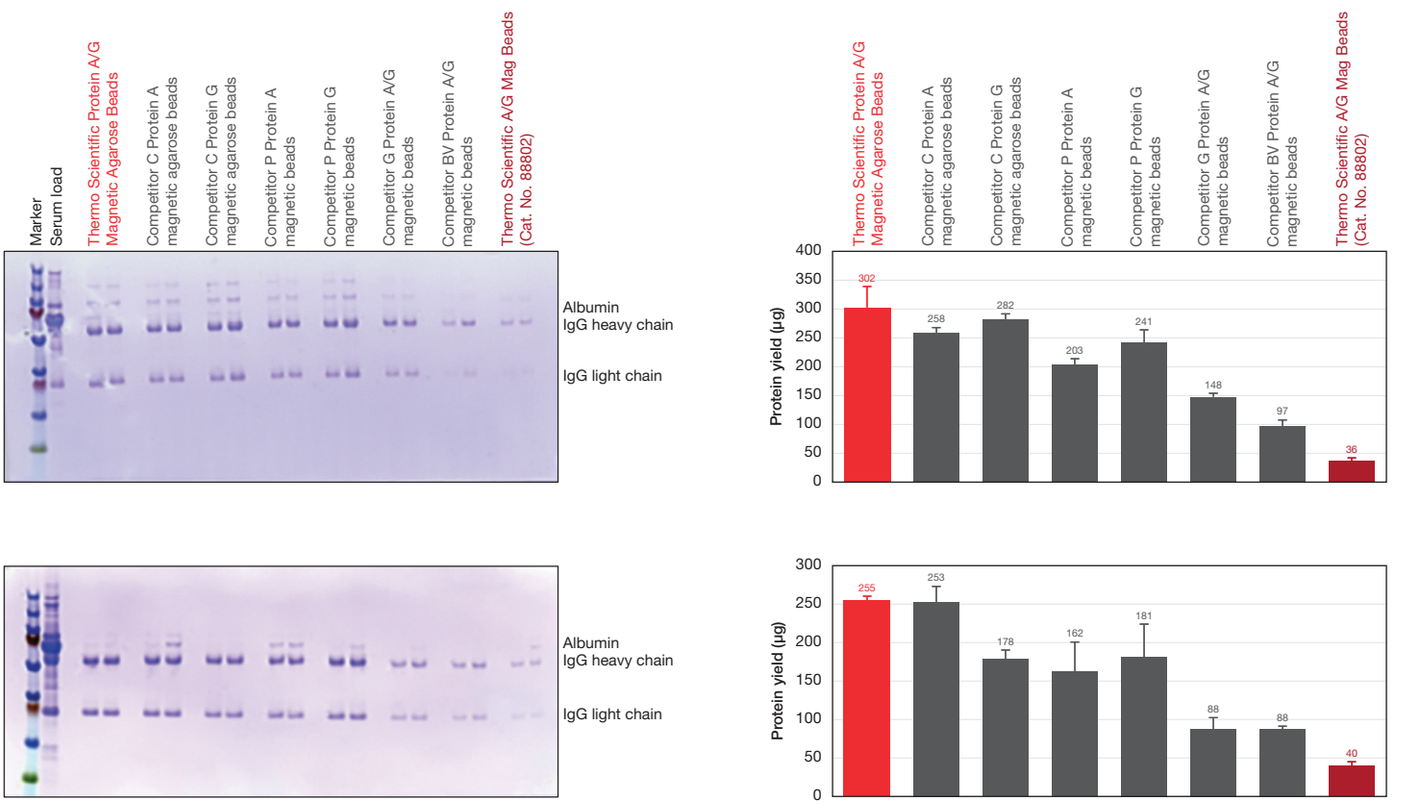
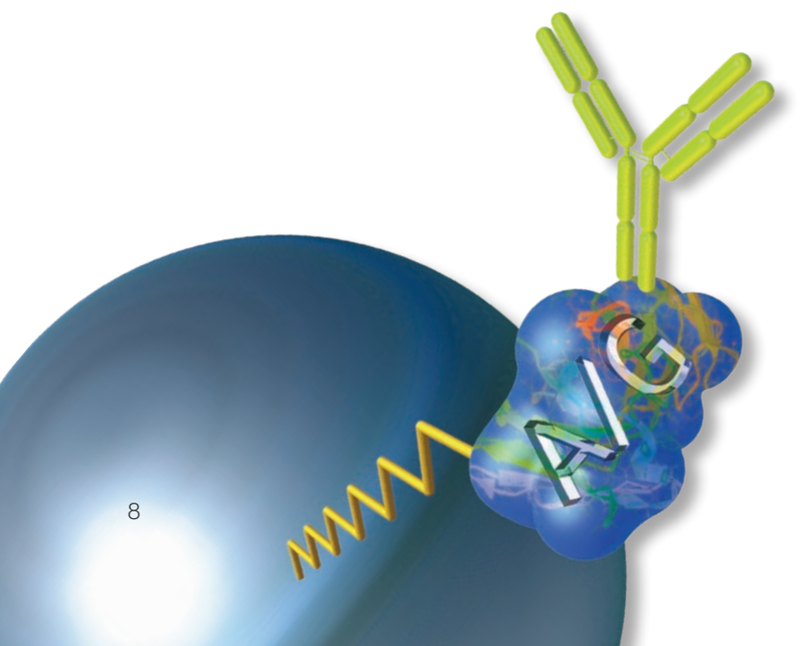


Figure 10. Thermo Scientific™ Pierce™ Protein A/G Magnetic Agarose Beads provide higher purification yields than other commercially available magnetic beads. IgG purification was performed with the Pierce Protein A/G Magnetic Agarose Beads, Protein A and Protein G magnetic agarose beads from competitor C, Protein A and Protein G magnetic beads from competitor P, Protein A/G magnetic beads from competitor G, Protein A/G magnetic beads from competitor BV, and Pierce Protein A/G Magnetic Beads. Mouse and human sera (50 µL) were diluted with binding buffer according to the manufacturer's protocol and added to magnetic agarose beads. IgG was purified following the manufacturer's protocol. IgG yield was estimated by absorbance of IgG at 280 nm. All purifications were done in duplicate.



Ordering information

Description	Quantity*	Cat. No
Pierce Protein A/G Magnetic Agarose Beads Sufficient for: Binding > 40 mg rabbit IgG/mL settled beads	1 mL	78609
Pierce Protein A/G Magnetic Agarose Beads Sufficient for: Binding > 40 mg rabbit IgG/mL settled beads	5 mL	78610

* Available in bulk quantities

Magnetic immunoprecipitation beads, cont.

Build your own immunoprecipitation assay

Thermo Scientific™ Pierce™ Protein A Magnetic Beads are used for immunoprecipitating antigens from cell or tissue extracts as well as purifying antibody from serum, cell culture supernatant or ascites fluid. Protein A can bind to antibodies from many different species, including mouse, human, rabbit, pig, dog and cat. The protocol for Pierce Protein A Beads is optimized for high recovery and high purity of isolated antibodies or antigens.

Highlights

- **Low nonspecific binding**—stable, pre-blocked beads provide clean purification product
- **Consistency**—magnetic beads eliminate resin loss and provide for more efficient separation of solutions than traditional IP methods that use only microcentrifuge tubes
- **Compatibility**—beads are compatible with manual and automated applications (e.g., KingFisher Instruments)

Table 4. Properties of Thermo Scientific Pierce Magnetic Protein A Beads.

Composition	Magnetite-coated polymeric beads blocked and covalently coated with a monolayer of recombinant Protein A
Mean Diameter	1 µm (nominal)
Density	2.0 g/cm^3
Bead Concentration	10 mg/mL in water with sodium azide
Binding Capacity	≥40 µg of rabbit IgG/mg of beads; ≥400 µg of rabbit IgG/mL of beads

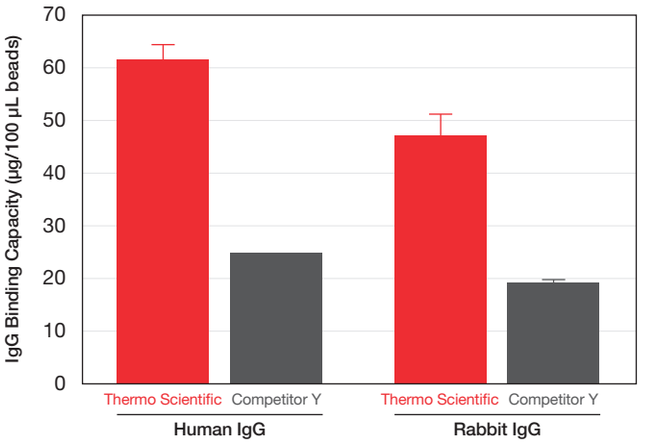


Figure 11. The human and rabbit IgG binding capacities of Thermo Scientific Pierce Protein A Magnetic Beads are approximately 2-fold higher than Protein A magnetic beads from competitor Y. Pierce Protein A Magnetic Beads or Protein A magnetic beads (competitor Y) were added to a 96 deep-well plate (100 µL beads per well). Using the KingFisher Flex Instrument, the beads were incubated for one hour with 400 µg purified human or rabbit IgG. Binding was calculated using the Pierce BCA Protein Assay by subtracting the amount of IgG in the flow-throughs from the IgG loaded.

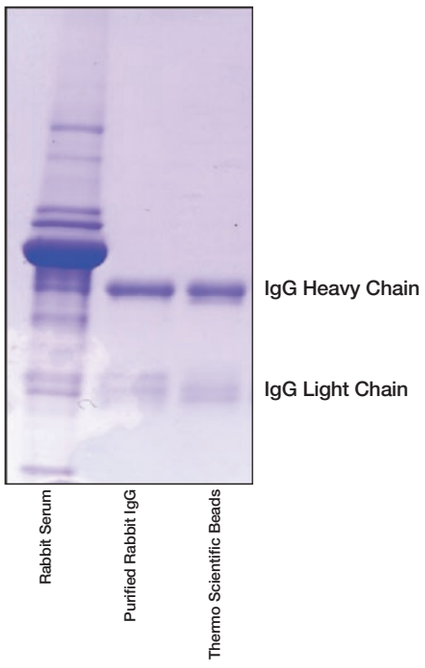


Figure 12. Thermo Scientific Pierce Protein A Magnetic Beads exhibit low nonspecific binding. Using a KingFisher Flex Instrument with a 96 deep-well plate, IgG was purified from 2 mg of rabbit serum using 50 µL of Pierce Protein A Magnetic Beads. The beads were incubated for one hour with serum diluted in phosphate-buffered saline containing 0.025% Tween-20 (PBST), washed twice with PBST and once with water, and then eluted with 0.1 M glycine, pH 2.0 for 10 minutes at room temperature. The eluates were resolved and stained with Imperial Protein Stain. No serum proteins other than antibody heavy and light chains were detected in the eluted sample.

Magnetic immunoprecipitation beads, cont.

Build your own immunoprecipitation assay

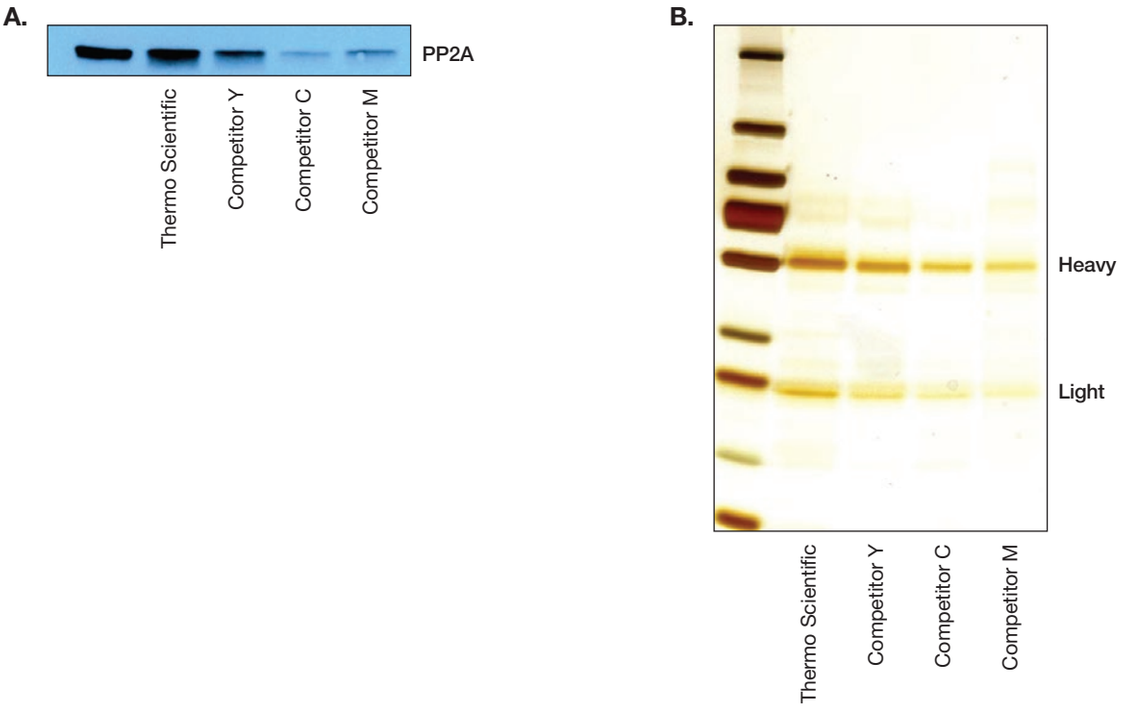
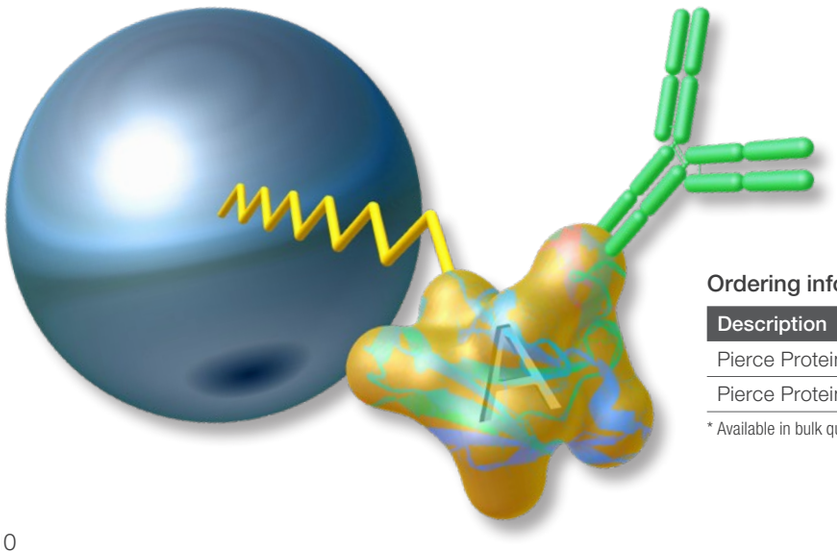


Figure 13. Better immunoprecipitation results with Thermo Scientific Pierce Protein A Magnetic Beads. PP2Aantibody (5 µg) was incubated overnight at 4˚C with 0.5 mg of A549 cell lysate. Using the KingFisher Flex Instrument, 50 µL each of Pierce Protein A Magnetic Beads, Protein A magnetic beads from competitors Y and competitor M, crosslinked, beaded-form of agarose Protein A magnetic bead from competitor C were added to 96 deep-well plates. The beads were incubated for one hour with the antigen/antibody complex at room temperature, washed twice in phosphate-buffered saline containing 0.05% Tween-20, washed once in water and then eluted in 0.1 M glycine, pH 2.0. Samples were resolved by SDS-PAGE and analyzed for Western blot for PP2A (Panel A) and by silver stain for nonspecific binding (Panel B). The Pierce Protein A Magnetic Beads were found to have higher yield of PP2A than other Protein A beads. Nonspecific binding was negligible for all beads tested.



Ordering information

Description	Quantity*	Cat. No
Pierce Protein A Magnetic Beads	1 mL	88845
Pierce Protein A Magnetic Beads	5 mL	88846

* Available in bulk quantities

Magnetic immunoprecipitation beads, cont.

Build your own immunoprecipitation assay

Thermo Scientific™ Pierce™ High-Capacity Protein A MagBeads, Alkali Stable, are high-performance magnetic beads for purification of antibodies from cell culture supernatant, ascites, serum, and crude extracts.

Highlights

- **High binding capacity**—≥ 40 mg HIgG/mL settled beads
- **Alkaline stable**—engineered to withstand standard CIP procedures with 0.1 M NaOH, enabling efficient cleaning of the beads for reuse
- **Zoofree**—Animal component-free raw materials
- **Versatile**—beads are compatible with manual and automated workflows (e.g., KingFisher instruments)
- **Stable**—high-affinity Protein A is securely immobilized to magnetic agarose to help prevent leaching of ligand, target antibody, or base support

The protein A ligand has been genetically modified for stability in the presence of NaOH, so the beads can be regenerated at least 10 times without sacrificing binding capacity. The ligand is produced in yeast and is immobilized on magnetic agarose with an average particle size of ~30 µm.

Pierce High-Capacity Protein A MagBeads, Alkali-Stable, are suitable for purifying mAbs and a subpopulation of scFv's and Fab fragments containing a VH3 domain. The beads are compatible with automation, allowing for higher throughput purifications and screening of purification conditions.

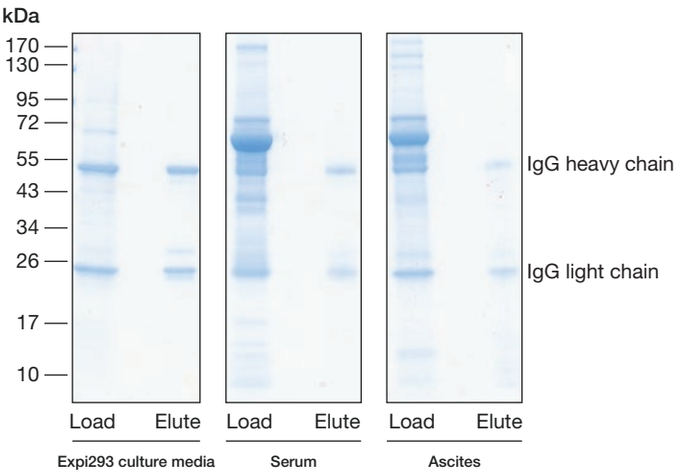


Figure 14. High purity IgG purification. Antibody was purified from Expi293 cell culture supernatant, human serum, and mouse ascites (IgG2b) using alkali-stable Pierce High-Capacity Protein A MagBeads (25 mL settled beads). Eluted fractions were separated by SDS-PAGE.

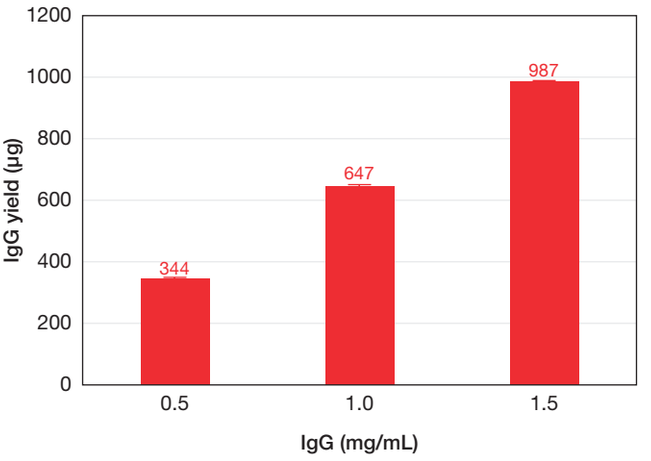


Figure 15. IgG purification from Expi293 Culture Medium. Expi293 supernatant containing over-expressed Humira antibody was purified at the concentrations shown using alkali-stable Pierce High-Capacity Protein A MagBeads (25 mL of settled beads) on a KingFisher Apex instrument, in duplicate. Beads were washed and then eluted with 0.1 M glycine, pH 2. Yield was determined by A280 absorbance of the eluates.

Magnetic immunoprecipitation beads, cont.

Build your own immunoprecipitation assay

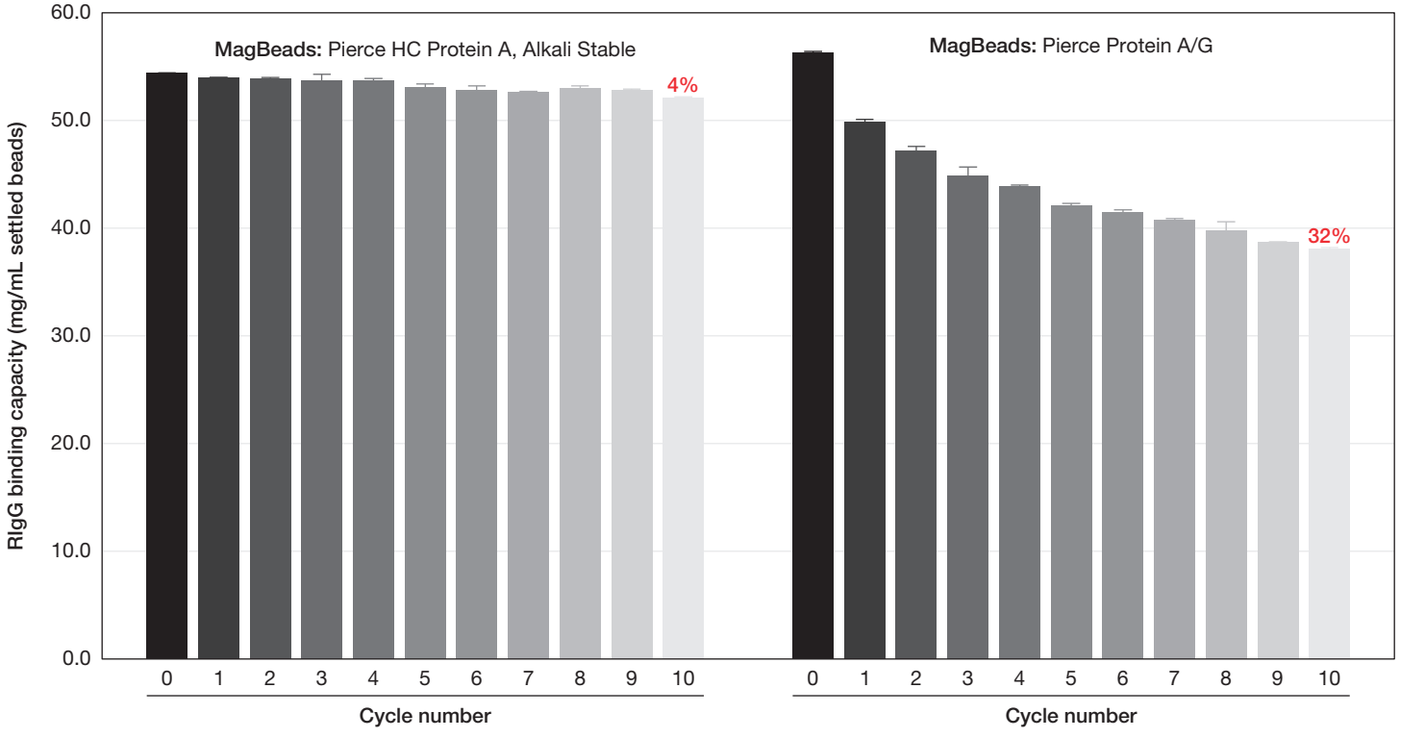


Figure 16. Stable magnetic bead regeneration using NaOH. Rabbit IgG (RIgG) was bound to Pierce High-Capacity Protein A MagBeads, Alkali Stable, and Pierce Protein A/G Magnetic Agarose, and the beads were eluted and regenerated with 0.1 M NaOH. RIgG binding capacity results over 10 cycles of regeneration are shown. Alkali-stable beads lost only 4% capacity compared with non-alkali-stable protein A/G beads, which lost 32% capacity. Reduction in yield was minimal with the alkali-stable beads, but significantly higher with the non-alkali-stable protein A/G beads.

Ordering information

Description	Quantity	Cat. No
Pierce™ High Capacity Protein A MagBeads, alkali stable Sufficient for: ≥40 mg HIgG/mL settled beads	1 mL	A53035
Pierce™ High Capacity Protein A MagBeads, alkali stable Sufficient for: ≥40 mg HIgG/mL settled beads	5 mL	A53036
Pierce™ High Capacity Protein A MagBeads, alkali stable Sufficient for: ≥40 mg HIgG/mL settled beads	25 mL	A53037
Pierce™ High Capacity Protein A MagBeads, alkali stable Sufficient for: ≥40 mg HIgG/mL settled beads	4 x 25 mL	A53038

Magnetic immunoprecipitation beads, cont.

Build your own immunoprecipitation assay

Thermo Scientific™ Pierce™ Protein G Magnetic Beads are high-capacity and high-throughput affinity particles for antibody purification and immunoprecipitation methods using manual or robotic magnetic separators. Protein G can bind to antibodies from many different species, including mouse, human, rabbit, cow, goat and sheep.

Highlights

- **High IP efficiency**—high antibody yield
- **Low nonspecific binding**—stable, pre-blocked beads provide clean purification product
- **Assay consistency**—magnetic beads eliminate resin loss
- **High throughput**—compatible with manual and automated applications (e.g., KingFisher Instruments)

Table 5. Properties of Thermo Scientific Pierce Magnetic Protein G Beads.

Composition	Magnetite-coated polymeric beads blocked and covalently coated with a monolayer of recombinant Protein G
Mean Diameter	1 µm (nominal)
Density	2.0 g/cm^3
Bead Concentration	10 mg/mL in water with sodium azide
Binding Capacity	≥60 µg of rabbit IgG/mg of beads; ≥600 µg of rabbit IgG/mL of beads

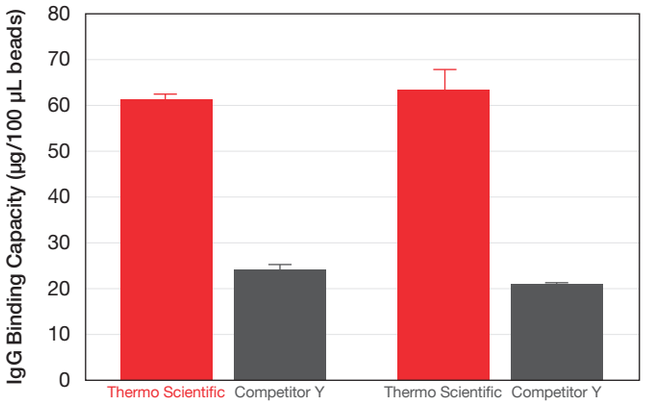


Figure 17. The human and rabbit IgG binding capacities of Thermo Scientific Pierce Protein G Magnetic Beads are approximately 3-fold higher than competitor Y magnetic beads. Pierce Protein G Magnetic Beads and competitor Y Protein G magnetic beads were added to a 96 deep-well plate (100 µL beads per well). Using the KingFisher Flex Instrument, the beads were incubated for one hour with 400 µg purified human or rabbit IgG. Binding was calculated using the Pierce BCA Protein Assay by subtracting the amount of IgG in the flow-throughs from the IgG loaded.

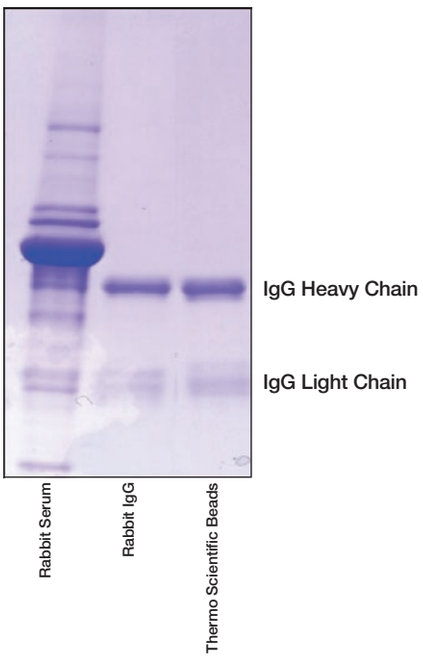


Figure 18. Thermo Scientific Pierce Protein G Magnetic Beads exhibit low nonspecific binding. Using a KingFisher Flex Instrument with a 96 deep-well plate, IgG was purified from 2 mg of rabbit serum using 50 µL of Pierce Protein G Magnetic Beads. The beads were incubated one hour with serum diluted in phosphate-buffered saline containing 0.025% Tween-20 (PBST), washed twice with PBST and once with water, and then eluted with 0.1 M glycine, pH 2.0 for 10 minutes at room temperature. The eluates were resolved and stained with Imperial Protein Stain. No serum proteins other than antibody heavy and light chains were detected in the eluted sample.

Magnetic immunoprecipitation beads, cont.

Build your own immunoprecipitation assay

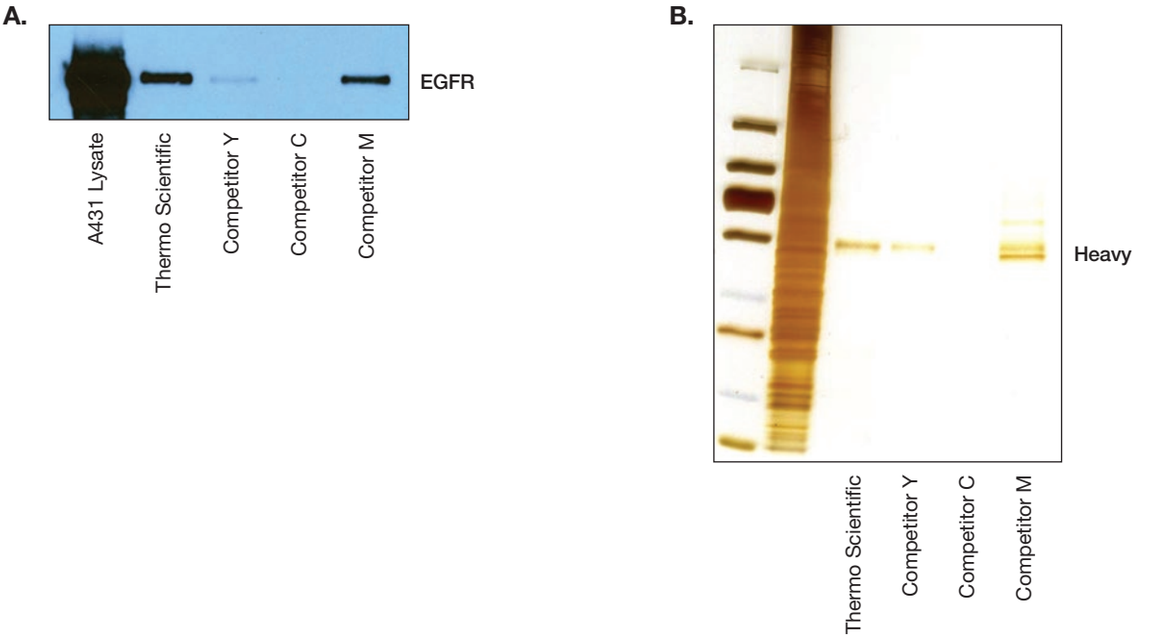
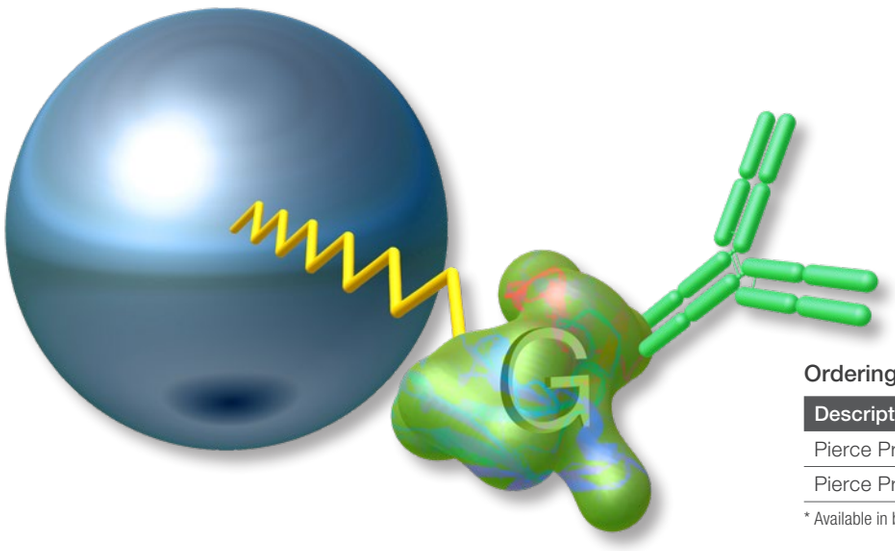


Figure 19. Better immunoprecipitation results with Thermo Scientific Pierce Protein G Magnetic Beads. EGF Receptor antibody (5 µg) was incubated overnight at 4˚C with 0.75 mg of A431cell lysate. Using the KingFisher Flex Instrument, 25 µL each of Pierce Protein G Magnetic Beads, Protein G magnetic beads from competitors Y and competitor M and crosslinked, beaded-form of agarose Protein G magnetic beads from competitor C were added to 96 deep-well plates. The beads were incubated for one hour with the antigen/antibody complex at room temperature, washed twice in phosphate-buffered saline containing 0.05% Tween-20, washed once in water and then eluted in 0.1 M glycine, pH 2.0. Samples were resolved by SDS-PAGE and analyzed by Western blot for EGFR (Panel A) and by silver stain for nonspecific binding (Panel B). The Pierce Protein G magnetic beads were found to have higher yield of EGFR than other Protein G beads. Nonspecific binding was negligible for all beads tested.



Ordering information

Description	Quantity*	Cat. No
Pierce Protein G Magnetic Beads	1 mL	88847
Pierce Protein G Magnetic Beads	5 mL	88848

* Available in bulk quantities

Magnetic immunoprecipitation beads, cont.

Build your own immunoprecipitation assay

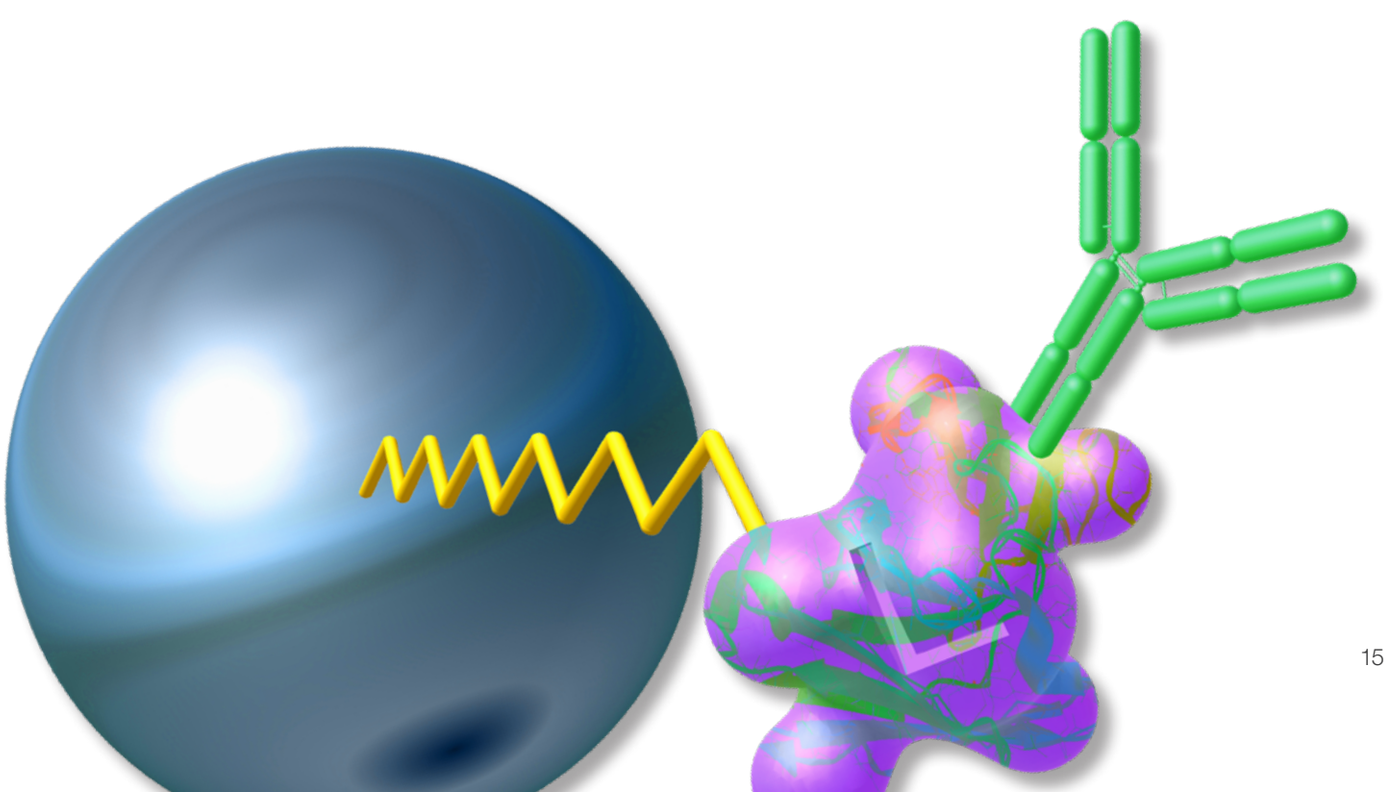
Thermo Scientific™ Pierce™ Protein L Magnetic Beads are ideal for selective isolation of antibodies possessing kappa light chains. Protein L selectively binds mouse and human antibodies through kappa light chains and is commonly used to purify monoclonal antibodies in cell culture supernantants supplemented with bovine serum as Protein L does not bind bovine IgG. Protein L can bind a broader range of Ig classes than Protein A or Protein G, including IgG, IgM, IgA, IgE and IgD. Protein L binds strongly to human (kappa I, III and IV only), mouse (kappa I only), rat and pig immunoglobulins. It binds weakly to rabbit immunoglobulins and does not bind to immunoglobulins from bovine, goat or sheep. Single-chain variable fragments (scFv) and Fab fragments also bind to Protein L.

Highlights

- **Selective**—optimal for selective purification of human and mouse antibodies that have kappa light chains
- **Low nonspecific binding**—stable, pre-blocked beads provide clean purification of antibody
- **Compatibility**—beads are compatible with manual and automated applications (e.g., KingFisher Instruments)

Table 6. Properties of Thermo Scientific Pierce Magnetic Protein L Beads.

Composition	Magnetite-coated polymeric beads blocked and covalently coated with a monolayer of recombinant Protein L
Mean Diameter	1 µm (nominal)
Density	2.0 g/cm^3
Bead Concentration	10 mg/mL in water with sodium azide
Binding Capacity	≥110 µg of human IgG/mg of beads; ≥1.1 mg of human IgG/mL of beads



Magnetic immunoprecipitation beads, cont.

Build your own immunoprecipitation assay

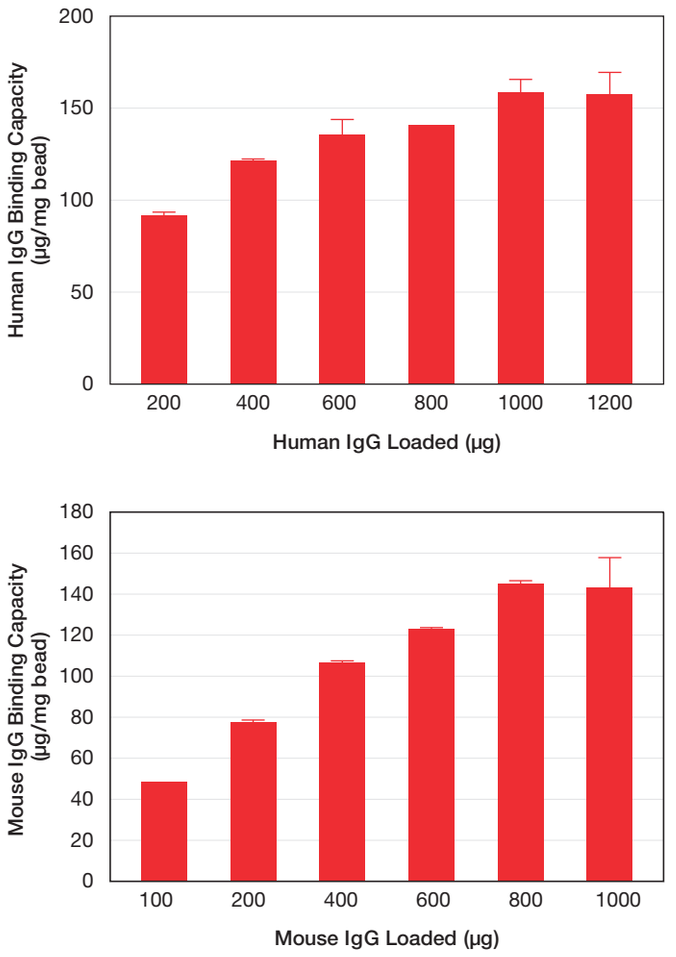


Figure 20. Human and mouse IgG binding capacity curves for Thermo Scientific Pierce Protein L Magnetic Beads. Pierce Protein L Magnetic Beads were added to a 96 deep-well plate (100 µL beads per well). Using the KingFisher Flex Instrument, the beads were incubated for one hour with purified human or mouse IgG (amounts shown in graphs). Binding was calculated using the Pierce BCA Protein Assay by subtracting the amount of IgG in the flow-throughs from the IgG loaded.

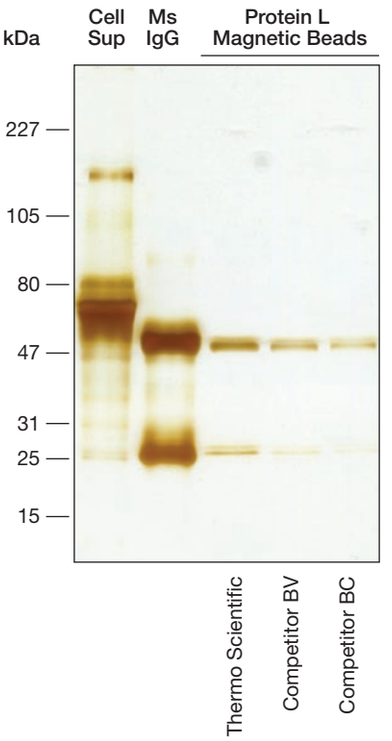


Figure 21. Thermo Scientific Pierce Protein L Magnetic Beads isolate more mouse IgG from cell culture supernatant than other suppliers' Protein L magnetic beads. Using a KingFisher Flex Instrument with 96 deep-well plates, IgG was purified from cell culture supernatant using 50 µL each of Pierce Protein L Magnetic Beads, and Protein L magnetic beads from competitors BV and BC. The beads were incubated for one hour with undiluted cell culture supernatant containing 0.025% Tween-20, washed twice with PBST and once with water, and then eluted with 0.1 M glycine, pH 2.0 for 10 minutes at room temperature. The eluates were resolved and stained with the Thermo Scientific Pierce Silver Stain Kit. Note that binding of mouse IgG with Protein L only occurs when kappa light chains are present. All of the beads were found to have negligible nonspecific binding.

Ordering information

Description	Quantity*	Cat. No
Pierce Protein L Magnetic Beads	1 mL	88849
Pierce Protein L Magnetic Beads	5 mL	88850

* Available in bulk quantities

Magnetic immunoprecipitation (IP) kits

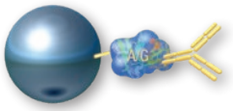
Select an easy-to-use validated kit

Thermo Scientific™ Pierce™ Magnetic IP/Co-IP Kits are optimized to isolate protein complexes from biological samples. Each kit contains all the required buffers and beads validated to deliver excellent results. Three versions of the kit are available to perform a classic IP, crosslink IP or direct IP.

Highlights

- Compatible with any antibody
- Faster immunoprecipitations (IPs) for less background
- Easily capture transient protein complexes
- No antibody contamination in your eluted sample
- Simple handling with no sample loss
- Validated for automated protocols using KingFisher Instruments

Thermo Scientific™ Pierce™ Classic Magnetic IP/Co-IP Kit



This kit uses high binding capacity Pierce Magnetic Protein A/G Beads to deliver clean and consistent co-immunoprecipitations (co-IP) with any common antibody. Antibodies are not linked to the resin and will co-elute with your antigen.

Select this version:

- For the highest antigen yield
- If antibody contamination is not a concern

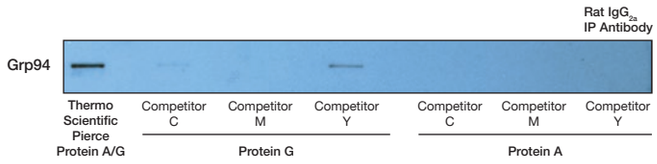


Figure 22. The Thermo Scientific Pierce Classic Magnetic IP/Co-IP Kit immunoprecipitates Grp94 with higher yield than other Protein A and Protein G beads. MOPC (mouse myeloma) cells were lysed in RIPA buffer and 0.75 mg of lysate was incubated with Grp94 antibody (rat IgG2a) overnight at 4°C. Using the KingFisher Flex Instrument, 50 µL of Pierce Protein A/G Magnetic Beads and 50 µL each of Protein A and Protein G Beads from competitor C, M and Y were added to a 96 deep-well plate. The eluates were resolved by SDS-PAGE and analyzed by Western blot for Grp94.

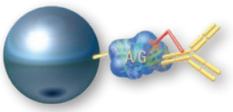
Ordering information

Description	Quantity	Cat. No
Pierce Classic Magnetic IP/Co-IP Kit Sufficient for: 40 IP reactions using 25 µL of beads Contains: Pierce Protein A/G Magnetic Beads, 1 mL Pierce IP Lysis/Wash Buffer, 2 x 50 mL Lane Marker Sample Buffer (5X), 5 mL Elution Buffer, 5 mL Neutralization Buffer, 0.5 mL	40-rxn kit	88804

Magnetic immunoprecipitation (IP) kits, cont.

Select an easy-to-use validated kit

Thermo Scientific™ Pierce™ Crosslink Magnetic IP/Co-IP Kit



This kit uses crosslinkers to immobilize your primary antibody to Protein A/G. This prevents antibody contamination in your eluted sample and eliminates antibody interference in Western blot and mass spec applications.

Select this version:

- To eliminate antibody contamination that interferes with downstream detection
- To properly orient your antibody

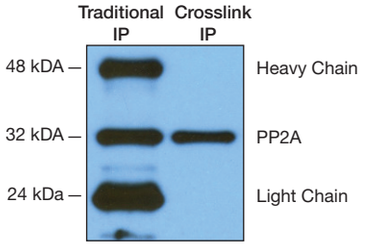
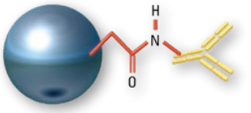


Figure 23. The Thermo Scientific Pierce Crosslink Magnetic IP/Co-IP Kit immunoprecipitates PP2A without antibody contamination and with negligible background. PP2A antibody (5 µg) was coupled to Pierce Protein A/G Magnetic Beads without DSS crosslinking (traditional IP) and with DSS crosslinking (crosslink IP). The beads were incubated with 0.5 mg of A549 cell lysate for one hour at room temperature on the KingFisher Flex Instrument. PP2A was eluted from the beads with elution buffer for five minutes at room temperature and then neutralized with neutralization buffer. The eluates, antibody control (Ab) and flow-through (FT) were resolved by SDS-PAGE and analyzed by Western blot for PP2A. The antibody-crosslinked Pierce Protein A/G Magnetic Beads effectively immunoprecipitated PP2A without antibody contamination whereas the traditional IP method resulted in significant antibody contamination in the eluate.

Ordering information

Description	Quantity	Cat. No
Pierce Crosslink Magnetic IP/Co-IP Kit Sufficient for: 40 IP reactions using 25 µL of beads Contains: Pierce Protein A/G Magnetic Beads, 1 mL IP Lysis/Wash Buffer, 2 x 50 mL Coupling Buffer (20X), 25 mL DSS Crosslinker, 8 x 2 mg Lane Marker Sample Buffer (5X), 5 mL Elution Buffer, 10 mL Neutralization Buffer, 1 mL Lane Marker Sample Buffer (5X), 5 mL	40-rxn kit	88805

Thermo Scientific™ Pierce™ Direct Magnetic IP/Co-IP Kit



This kit uses Pierce NHS-Activated Magnetic Beads to immobilize your primary antibody directly to the bead surface. This method is independent of antibody species and prevents antibody contamination in your eluted sample.

Select this version:

- For non-traditional antibodies that do not bind Protein A or Protein G
- To eliminate antibody contamination

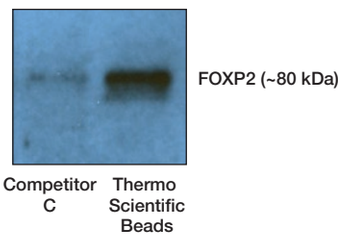


Figure 24. Better immunoprecipitation results with Thermo Scientific Pierce NHS-Activated Magnetic Beads. Anti-FOXP2 antibody (5 µg) was coupled to 25 µL of Pierce NHS-Activated Magnetic Beads and an equivalent amount of crosslinked, beaded-form of agarose NHS magnetic beads (competitor C). The two sets of prepared beads were then used to immunoprecipitate FOXP2 from 0.5 mg aliquots of the same 293T (human epithelial kidney) cell lysate. The eluates were resolved by SDS-PAGE and analyzed by Western blot for FOXP2.

Ordering information

Description	Quantity	Cat. No
Pierce Direct Magnetic IP/Co-IP Kit Sufficient for: 40 IP reactions using 25 µL of beads Contains: Pierce NHS-Activated Magnetic Beads, 1 mL IP Lysis/Wash Buffer, 2 x 50 mL Elution Buffer, pH 2.0, 5 mL Lane Marker Sample Buffer, Non-reducing, (5X), 5 mL Neutralization Buffer, pH 8.5, 0.5 mL 0.67 M Borate Buffer, 1 mL BupH Borate Buffer Pack, 1 pack Quenching Buffer, 25 mL	40-rxn kit	88828

Magnetic anti-HA IP kit

Select an easy-to-use validated kit

The Thermo Scientific™ Pierce™ HA-Tag Magnetic IP/Co-IP Kit provides an easy and fast method to study protein interactions. The high affinity anti-HA antibody-coupled magnetic beads enables immunoprecipitation (IP) of HA-tagged proteins or co-immunoprecipitation (co-IP) of their interacting partners without antibody contamination.

Highlights

- **Specific**—immunoprecipitate only HA-tagged proteins and their interactors
- **Validated**—IP/co-IP kit includes a positive control lysate
- **Robust**—compatible with common tissue culture cell lysates
- **Convenient and easy**—complete kit includes all necessary reagents to perform 40 reactions

Table 7. Properties of Thermo Scientific Pierce Anti-HA Magnetic Beads.

Composition	Magnetite-coated polymeric beads blocked and covalently coated with a mouse monoclonal IgG_1 anti-HA antibody
Mean Diameter	1 µm (nominal)
Density	2.0 g/cm^3
Bead Concentration	10 mg/mL in water with sodium azide
Binding Capacity	>10 µg of HA-tagged protein/mg beads (70 kDA)

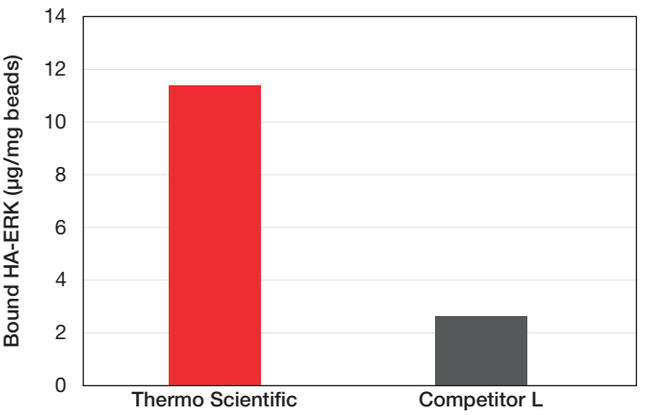


Figure 25. Significantly higher binding capacity with Thermo Scientific Pierce Anti-HA Magnetic Beads. HA-tagged protein (HA-ERK-GST, 400 µg in PBS) was incubated with 100 µL each of Thermo Scientific Pierce Anti-HA Magnetic Beads or competitor L Anti-HA magnetic beads for one hour at room temperature. Bound HA-tagged protein was measured using the Pierce BCA Assay. Pierce Anti-HA Magnetic Beads pulled down more than four times as much protein as the equivalent amount of competitor L Anti-HA magnetic beads.

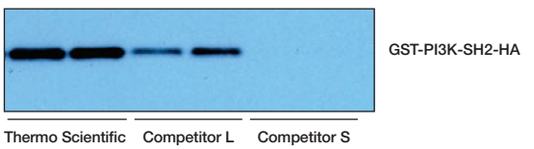


Figure 26. Better Immunoprecipitation results from *E. coli* lysates. Using a KingFisher Flex Instrument with 96 deep-well plates, 25 µL each of Pierce Anti-HA Magnetic Beads, Anti-HA-tag magnetic beads (competitor L) and Rabbit Anti-HA magnetic beads (competitor S) were used to immunoprecipitate GST-PI3K-SH2-HA from 50 µg of *E. coli* lysate in duplicate. Captured protein was eluted with 0.1 M glycine, pH 2.0, and then resolved by SDS-PAGE and analyzed by Western blot for the HA-tagged protein.

Magnetic anti-HA IP kit, cont.

Select an easy-to-use validated kit

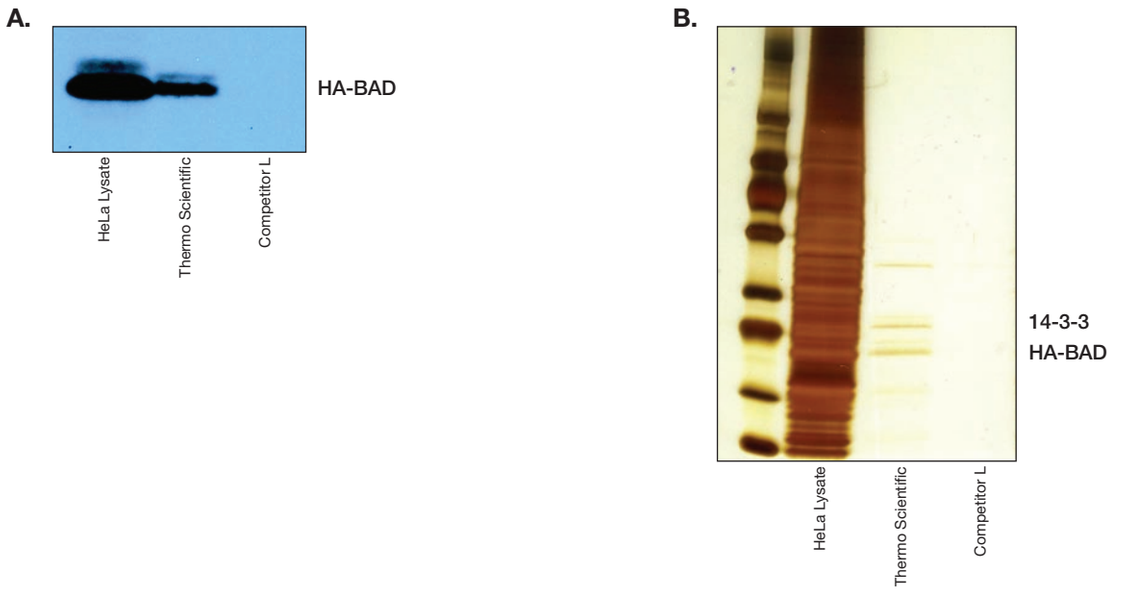


Figure 27. Better immunoprecipitation of protein expressed *in vitro*. Using the KingFisher Flex Instrument, 25 µL each of Pierce Anti-HA Magnetic Beads and Anti-HA-tag magnetic beads (competitor L) were added to 96 deep-well plates. The beads were incubated for one hour with HA-tagged BAD protein, expressed using the Thermo Scientific 1-Step High-Yield *In vitro* Translation Kit. After one hour at room temperature, samples were washed twice in phosphate-buffered saline containing 0.05% Tween-20, washed once in water and then eluted in 0.1 M glycine, pH 2.0. Samples were resolved by SDS-PAGE and analyzed by Western blot for HA (Panel A) and by silver stain for nonspecific binding (Panel B). HA-BAD fusion protein yield was highest with Pierce Anti-HA Magnetic Beads compared to other anti-HA beads. Nonspecific binding was negligible and there was co-immunoprecipitation of the protein 14-3-3.

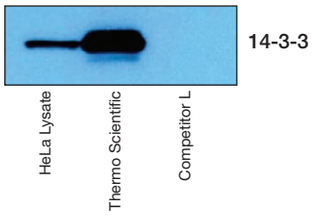


Figure 28. Better co-IP results with Thermo Scientific Pierce Anti-HA Magnetic Beads. Serine phosphorylation of BAD is associated with 14-3-3 binding and inhibition of BAD-induced cell death. Using a magnetic stand, 50 µL each of Pierce Anti-HA Magnetic Beads and Anti-HA-tag magnetic beads (competitor L) were added to microcentrifuge tubes. The beads were incubated for one hour at room temperature with HA-tagged BAD expressed in the 1-Step Human High-Yield IVT Kit. After incubation, the beads were washed twice in phosphate-buffered saline containing 0.05% Tween-20, washed once in water and then eluted in 30% acetonitrile/0.5% formic acid. Samples were dried down in a speedvac and brought back up in 50 µL of reducing SDS-PAGE samples buffer. One half of the reconstituted eluate was resolved by SDS-PAGE and analyzed by Western blot for 14-3-3. Pierce Anti-HA Magnetic Beads were found to have higher yield of 14-3-3 than the other anti-HA beads. Nonspecific binding was negligible.

Ordering information

Description	Quantity*	Cat. No
Pierce Anti-HA Magnetic Beads	1 mL	88836
Pierce Anti-HA Magnetic Beads	5 mL	88837
Pierce HA-Tag Magnetic IP/Co-IP Kit Sufficient For: 40 IP reactions using 25 µL of anti-HA magnetic beads Kit Contents: HA-tagged Positive Control (Cat. No 26180X), 500 µL Application Set (Cat. No 88838X): Pierce Anti-HA Magnetic Beads, 0.65 mL Pierce IP Lysis/Wash Buffer, 2 x 50 mL, pH 7.4 Lane Marker Sample Buffer, Non-reducing, (5X), 5 mL, pH 6.8 Elution Buffer, 5 mL, pH 2.0 Neutralization Buffer, 1 mL, pH 8.5	40-rxn kit	88838

Magnetic c-Myc-Tag IP Kit

Select an easy-to-use validated kit

The Thermo Scientific™ Pierce™ Magnetic c-Myc-Tag IP/Co-IP Kit contains specific immunoaffinity magnetic beads and reagents to perform immunoprecipitation assays of c-Myc fusion proteins or co-IP experiments using c-Myc-tagged bait proteins.

Unlike traditional IP procedures based on capture with Protein A/G beads, this kit uses magnetic beads containing pre-immobilized anti-c-Myc antibody. These Pierce Anti-c-Myc Magnetic Beads ensure specific binding of c-Myc tagged protein complexes from biological samples. Because the antibody is covalently attached to the beads, IP-targets are easily eluted and recovered without antibody contamination. The complete IP kit includes the magnetic beads, lysis/wash buffer, low-pH elution buffer, neutralization buffer, c-Myc-tag positive control lysate, and non-reducing sample buffer for SDS-PAGE. Protocols are provided for both manual and automated magnetic separation workflows. Sufficient components are provided to perform 40 IP or co-IP assays.

Highlights

- **Specific magnetic beads**—covalently immobilized high-quality anti-c-Myc monoclonal antibody enables high yields of immunoprecipitation products
- **Low non-specific binding**—stable, pre-blocked beads and specific antibody minimize off-target binding
- **Trouble-free elution**—low-pH elution buffer ensures recovery of c-Myc-tagged protein interaction complexes without antibody leaching contamination
- **Convenient and fast**—complete kit and easy-to-follow instructions provide optimized protocols to perform IP or Co-IP experiment in approximately 1 hour
- **Versatile**—magnetic beads are compatible with manual and automated magnetic separation workflows (e.g., Thermo Scientific KingFisher Instruments)

The c-Myc peptide (EQKLISEEDL) derived from the C-terminus region of human c-Myc protein is one of several fusion protein tags used for recombinant protein expression. The Pierce c-Myc Magnetic IP/Co-IP Kit uses a specific, high-affinity immobilized antibody (clone 9E10) for rapid immunoprecipitation of c-Myc tagged fusion proteins from bacterial and mammalian cell lysates, as well as from lysates prepared with the Pierce Human in vitro Translation Kits. The beads are incubated with a cell lysate containing c-Myc tagged protein, the fusion protein is captured, and the beads are subsequently washed and then eluted using low-pH elution buffer or non-reducing sample buffer. The protocol and buffers have been optimized for both IP and co-IP reactions, enriching for specific protein interaction complexes in the eluted samples. Anti-c-Myc antibody can be used to detect c-Myc tagged protein by Western blot analysis.

Product characteristics

Table 8. Characteristics of Thermo Scientific Pierce Anti-c-Myc Magnetic Beads.

Composition	High-affinity mouse IgG1 monoclonal antibody (clone 9E10) covalently coupled to the surface of blocked magnetic beads
Magnetization	Superparamagnetic (no magnetic memory)
Mean Diameter	1 µm (nominal)
Density	2.0 g/cm^3
Bead concentration	10 mg/mL
Binding capacity	≥10 µg of GST-c-Myc (26 kDa fusion protein)/mg of beads; ≥100 µg of GST-c-Myc (26 kDa fusion protein)/mL of bead suspension

Magnetic c-Myc-Tag IP Kit, cont.

Select an easy-to-use validated kit

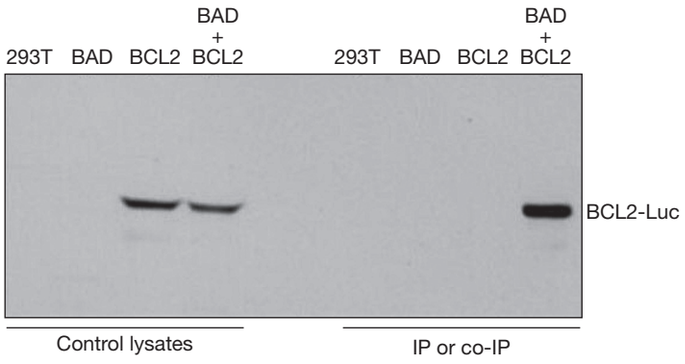


Figure 29. Co-IP of BCL2-Luc with c-MYC-tagged BAD in 293T cells. This experiment tests the specificity of co-immunoprecipitation by comparing results from 293T cells cultured alone (293T) or with vectors expressing BCL2-Luc and/or BAD-c-Myc fusion proteins (labeled BCL2 and BAD, respectively). The Western blot reveals the presence of BCL2 detected through its fused luciferase domain (i.e., via an anti-Luc antibody). Although BCL2 is expressed in two cultures (as indicated by its detection in two of the Control Lysates), it is purified by anti-c-Myc magnetic beads (IP or Co-IP lanes) only when co-expressed and captured through its interaction with c-Myc-tagged BAD (BAD+BCL2 lane). For the IP experiments, lysates were incubated with anti-c-Myc magnetic beads (50 µL) for two hours at 4°C, then processed and eluted using the kit components and procedure.

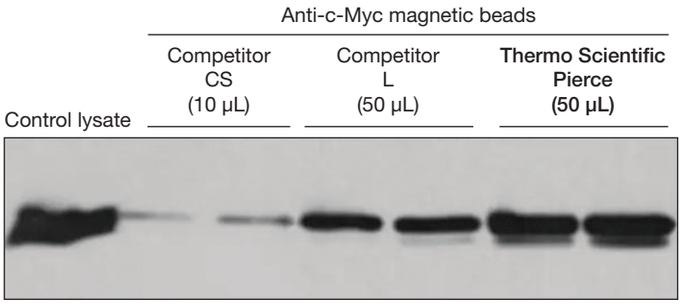


Figure 30. Better immunoprecipitation results with anti-c-Myc magnetic beads. Green Renilla luciferase c-Myc fusion protein was expressed in 293T cells. For IP, identical aliquots of the cell lysate were incubated in duplicate for one hour at room temperature with anti-c-Myc magnetic beads from each manufacturer. For all conditions, IP products were eluted identically using low-pH buffer. Eluted fractions (25 µL each) were separated by 12% SDS-PAGE, transferred to PVDF membrane, and detected via anti-c-Myc antibody (Part No. MA1-980), goat anti-mouse secondary antibody, and chemiluminescent substrate (Part No. 34080). Note: Manufacturers supply magnetic beads slurries at different concentrations and recommend different amounts of beads per microgram of lysate for IP. For this reason, only 10 µL of CS beads were used in this experiment. Nevertheless, Pierce beads provide equal or greater binding capacity, even in experiments involving carefully matched numbers of beads (data not shown).

Ordering information

Description	Quantity	Cat. No
Pierce Anti-c-Myc Magnetic Beads	1 mL	88842
Pierce Anti-c-Myc Magnetic Beads	5 mL	88843
Pierce c-Myc-Tag Magnetic IP/Co-IP Kit Sufficient For: 40 IP reactions using 25 µL of anti-c-Myc magnetic beads Kit contents: c-Myc-tagged Positive Control (Part No. 23633), 500 µL Pierce Anti-c-Myc Magnetic Beads, 1 mL Mag c-Myc IP/Co-IP Buffer-1, 50 mL Mag c-Myc IP/Co-IP Buffer-2 (20X), 20 mL Non-Reducing Sample Buffer (5X), 5 mL Elution Buffer (pH 2.0), 5 mL	40-rxn kit	88844

Magnetic ChIP kit

Select an easy-to-use validated kit

The Thermo Scientific™ Pierce™ Magnetic ChIP Kit provides an easy, fast and reproducible method to perform chromatin immunoprecipitation (ChIP) assays to capture a snapshot of specific protein-DNA interactions as they occur in living cells and then quantitate the interactions using PCR.

The Pierce Magnetic ChIP Kit contains sufficient reagents to perform 30 ChIP assays with appropriate controls using an optimized protocol. The blocked Pierce Protein A/G Magnetic beads used in this kit provide high binding capacity, low nonspecific background and flexibility of antibody species. These beads can be used manually with a magnetic stand as well as with automated platforms such as KingFisher Instruments. This kit provides reagents and a method to capture protein-DNA interactions in vivo allowing relative protein binding events to be monitored under different conditions and/or treatments. ChIP-validated and quality-guaranteed antibodies are also available for use with the Pierce Magnetic ChIP Kit.

Highlights

- **Easy and fast**—obtain purified DNA ready for PCR in about eight hours
- **Efficient and reproducible**—micrococcal nuclease digestion and nuclear lysis are highly optimized
- **Sensitive**—obtain results with as little as 1 × 10^4 cells
- **Low nonspecific background**—Pierce Protein A/G Magnetic beads are blocked in a non-DNA-containing reagent to minimize background
- **Complete**—optimized positive control reagents are included: RNA polymerase II antibody and GAPDH promoter PCR primers

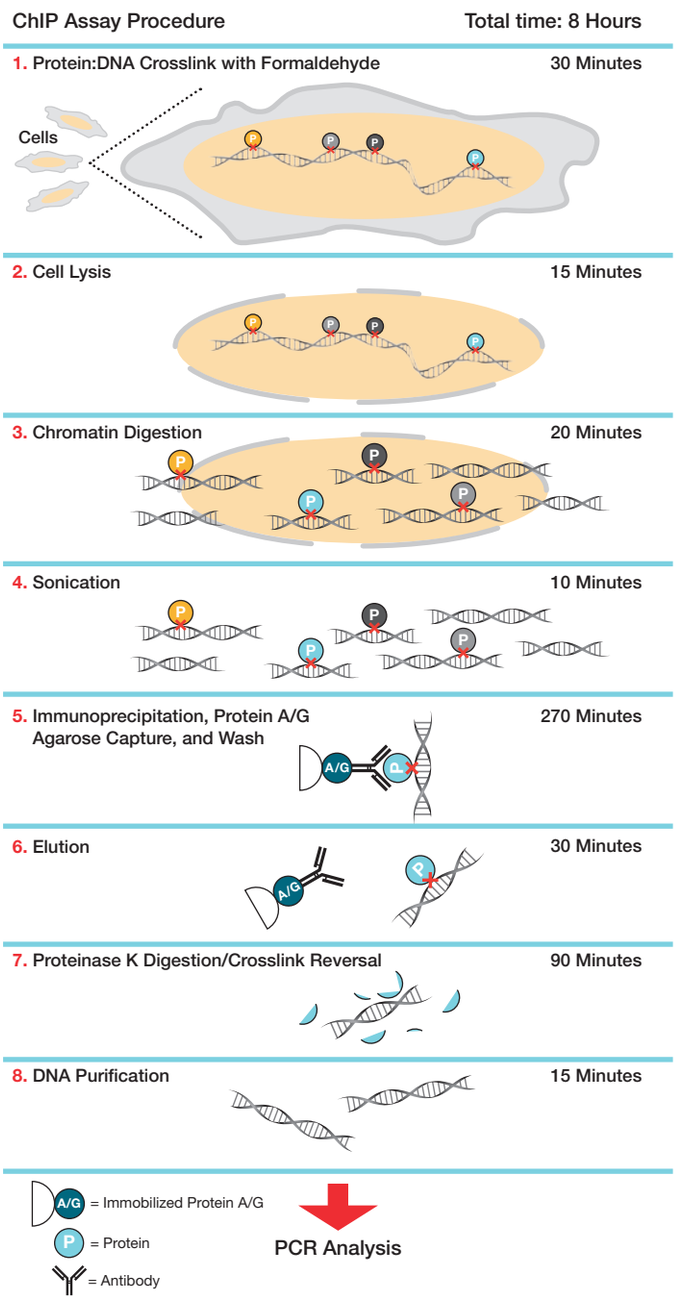


Figure 31. Overview of the Thermo Scientific Pierce Magnetic ChIP Kit protocol. The Pierce Magnetic ChIP assay protocol and reagents provide an optimized system for performing chromatin crosslinking, cell lysis, IP and target protein recovery about eight hours.

Magnetic ChIP kit, cont.

Select an easy-to-use validated kit

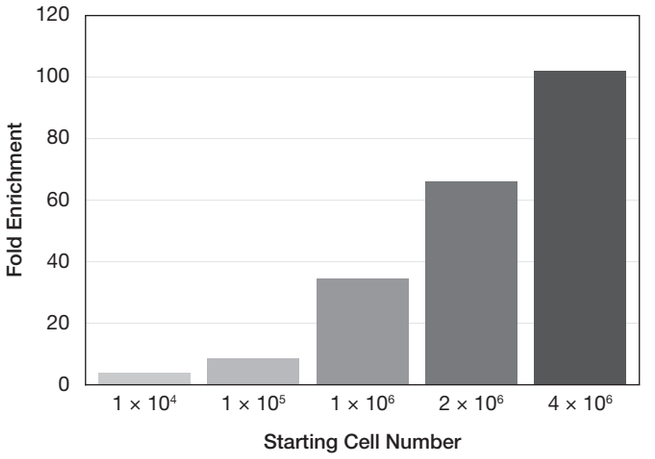


Figure 32. The Thermo Scientific Pierce Magnetic ChIP Kit has a broad range of sensitivity. A431 lung carcinoma cells were crosslinked using a final concentration of 1% formaldehyde for 10 minutes. ChIP assays were performed with the Pierce Magnetic ChIP Kit to determine binding of RNA polymerase II to the proximal GAPDH promoter. Quantitative real-time PCR data was obtained with a Bio-Rad iQ5 Thermocycler. Each column represents the fold enrichment of the RNA polymerase II over the normal rabbit IgG using the noted starting cell number (i.e., chromatin from that number of cells).

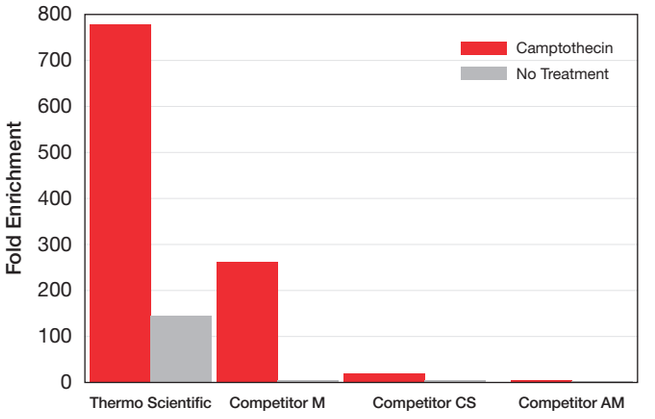


Figure 33. Greater fold enrichment than other kits. LNcaP prostate carcinoma cells were cultured in RPMI-1640 containing 10% FBS for 24 hours. Half of the cultures plated were treated for 16 hours with 5 µM camptothecin, a drug that inhibits DNA topoisomerase I. Crosslinking was achieved using a final concentration of 1% formaldehyde in the media for 10 minutes. ChIP assays were performed according to the manufacturers' protocols to determine binding of p53 to a 1.5-kb region of the CDKN1A (p21) promoter. Quantitive real-time PCR data was obtained with a Bio-Rad iQ5 Thermocycler. The Pierce Magnetic ChIP Kit has been optimized to isolate even large DNA fragments.

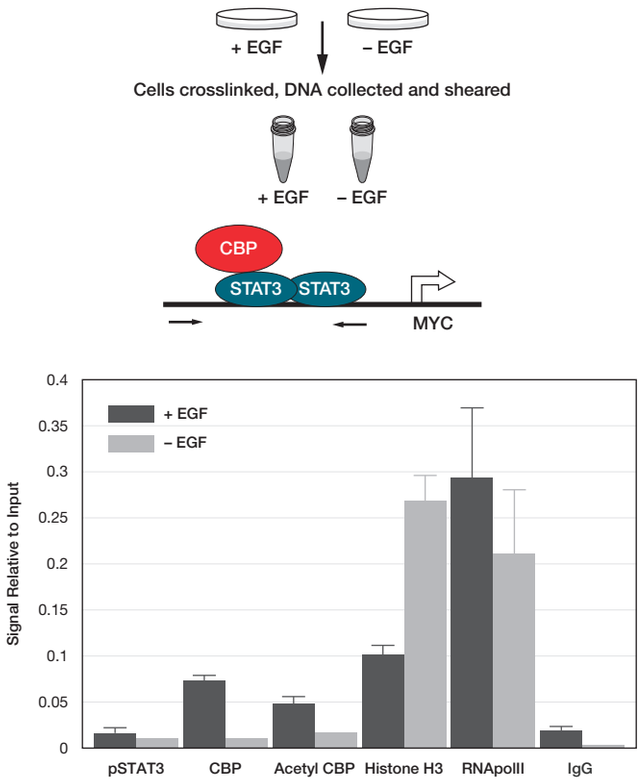


Figure 34. Profiling multiple transcription factors binding to the MYC promoter using the Thermo Scientific Pierce Magnetic ChIP Kit. A431 lung carcinoma cells were cultured in DMEM containing 10% FBS for 24 hours. Following a 24-hour serum withdrawal, half of the cultures plated were treated with 100 ng/mL EGF for 10 minutes. Crosslinking was achieved using a final concentration of 1% formaldehyde in the media for 10 minutes. ChIP assays were performed with the Pierce Magnetic ChIP Kit to determine binding of phosphorylated-STAT3, CBP, acetylated-CBP, histone H3, and RNA polymerase II to the proximal MYC promoter. Primary antibody amounts were determined empirically. Quantitive real-time PCR data was obtained with a Bio-Rad iQ5 Thermocycler. Graph represents the signal relative to the total input of chromatin.

Ordering information

Description	Quantity	Cat. No
Pierce Magnetic ChIP Kit Sufficient reagents to perform 30 ChIP Reactions	30-rxn kit	26157
ChIP-grade Protein A/G Magnetic Beads Formulation: Magnetite- and protein-coated polymer beads at 10 mg/mL in water with sodium azide. Sufficient For: Use in approx. 250 typical ChIP assays	5 mL	26162

Magnetic RNA-Protein Pull-Down Kit

Select an easy-to-use validated kit

The Thermo Scientific™ Pierce™ Magnetic RNA-Protein Pull-Down Kit provides researchers with a streamlined, robust method for enrichment and identification of RNA binding proteins. This method uses RNA probes labeled at the 3'- end with desthiobiotin and magnetic streptavidin particles. The complete kit contains sufficient reagents for 20 RNA-labeling reactions and 20 RNA-protein pull-down assays. Both synthetic RNA or *in vitro* transcribed RNA can be labeled with desthiobiotin. RNA-binding proteins are then enriched from cellular/tissue lysates or from *in vitro* translated protein preps. RNA-binding proteins are detected using Western blotting or mass spectrometry (MS).

Highlights

- **Direct**—capture ribonucleoprotein complexes directly from cell lysates
- **Easy to use**—magnetic beads enable easy processing for multiple samples
- **Flexible**—enrich RNA binding proteins from cell/tissue lysates or *in vitro* translated protein preps
- **Clean**—magnetic format yields low background
- **Specific**—perform RNA mutations to map interaction sites
- **Complete**—contains both labeling and enrichment modules with buffers necessary for assay; positive control RNA, negative control RNA and anti-RBP antibody included

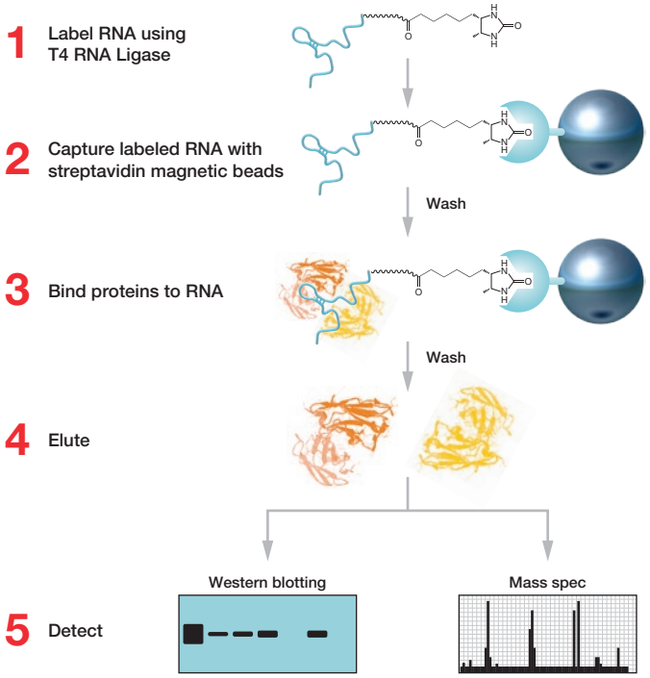


Figure 35. Easy RNA labeling and interaction analysis. RNA probes are first labeled at the 3' end with desthiobiotin using T4 RNA Ligase. RNA probes are then immobilized onto magnetic streptavidin particles and incubated with protein from cell lysates or *in vitro* translation preps. RNA-binding proteins are eluted and detected by Western blotting or mass spec.

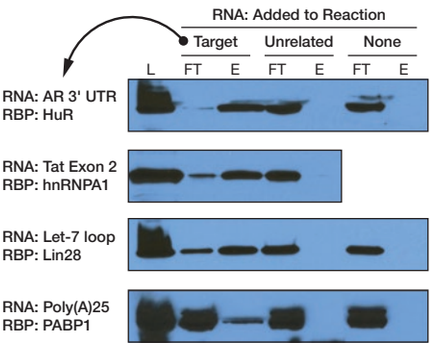


Figure 36. End-labeled RNA enriches specific target binding proteins. RNA binding proteins (RBP) of the AR 3' UTR control system (top panel) and three experimental systems were enriched according to kit procedure. L = lysate; FT = flow-through; E = elute.

Incubation of A431 lysate with labeled AR UTR RNA (Target) enriches HuR RBP, while incubation with negative control poly(A) RNA (Unrelated) or beads only (None) does not (compare elution lanes). The same pattern results with the experimental systems, confirming the proper function of the kit. Samples were normalized by volume, and bands were detected using Thermo Scientific SuperSignal West Pico Substrate by a 2-minute exposure to film. Target RNA sequences were as follows:

Androgen Receptor 3' UTR (Kit Control System):
5'-CUGGGCUUUUUUUUUCUCUUUCUCUCCUUUCUUUUUCUUCUUCCCUCCCUA-3'

Tat Exon 2:
5'-UUACUCAACAGAGGAGAGCAAGAAAUGGAGCCAGUAGAUCCUAGACUAGAGCCCUGG-3'

Let-7 loop:
5'-CAGUUUGAGGGUCUAUGAUACCACCGGUACAAGAUAACUG-3'

Poly(A)25:
5'-AAAAAAAAAAAAAAAAAAAAAAAAA-3'

Magnetic RNA-Protein Pull-Down Kit, cont.

Select an easy-to-use validated kit

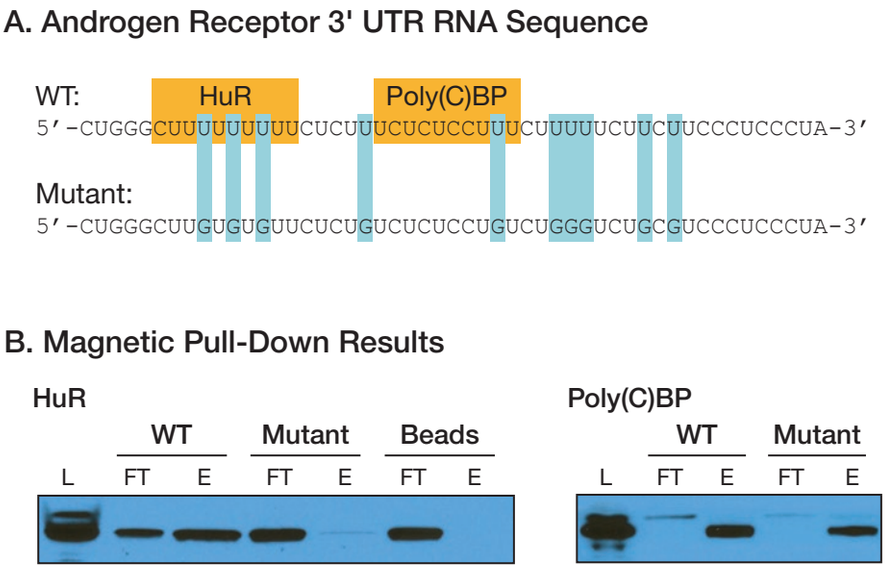


Figure 37. Androgen receptor 3'-UTR RNA specifically pulls-down HuR. Wild-type and mutant AR 3'-UTR RNA (50pmol) were labeled using cytidine bisphosphate desthiobiotin and T4 RNA ligase. Labeled RNA was captured using 50µL of streptavidin magnetic beads in RNA capture buffer for 30 minutes at room temperature. Beads were washed twice in 20mM Tris (pH 7.5), once in Protein-RNA binding buffer, and 40 µg of A431 extract was added. Samples were incubated 45 minutes at 4˚C, washed three times with wash buffer and eluted after 15 minutes of incubation at 37˚C with elution buffer. RNA pull-down specificity was assessed by Western blotting. Samples were normalized by volume and bands were detected using SuperSignal West Pico Substrate. Exposure time = 2 minutes. L – lysate load; FT – flow-through, E – elute. PCBP1 antibody (1:1000, Genway Biotech #GWB-38F6F3).

Magnetic RNA-Protein Pull-Down Kit, cont.

Select an easy-to-use validated kit

MS Peptide Identification

ELAV1_HUMAN (100%), 38,092.6 Da
ELAV-like protein 1 OS=Homo sampiens GN=ELAVL1 PE=1 SV=2
2 unique peptides, 2 unique spectra, 2 total spectra, 22/329 amino acids (7% coverege)

MSNGYEDHMA EDCRGDIGRT NLIVNYLPQN MTQDELRSLF SSIGEVESAK
LIRDKVAGHS LGYGFVNYVT AKDAERAINT LNGLRLQSKT IKVSYARPSS
EVIKDANLYI SGLPRTMTQK DVEDMFSRFG RIINSRVLVD QTTGLSRGVA
FIRFDKRSEA EEAITSFNGH KPPGSSEPIT VKFAANPNQN KNVALLSQLY
HSPARRFGGP VHHQAQRFRF SPMGVDHMSG LSGVNVPGNA SSGWCIFIYN
LGQDADEGIL WQMFGPFGAV TNVKVIRDFN TNKCKGFGFV TMTNYEEAAM
AIASLNGYRL GDKILQVSFK TNKSHK

PCB8_HUMAN (100%), 38,580.9 Da
Poly(rC)-binding protein 2 OS=Homo sampiens GN=PCBP2 PE=1 SV=1
6 unique peptides, 10 unique spectra, 11 total spectra, 87/365 amino acids (24% coverege)

MDTGVIEGGL NVTLTIRLLM HGKEVGSIIG KKGESVKKMR EESGARINIS
EGNCPERIIT LAGPTNAIFK AFAMIIDKLE EDISSSMTNS TAASRPPVTL
RLVVPASQCG SLIGKGGCKI KEIRESTGAQ VQVAGDMLPN STERAITIAG
IPQSIIECVK QICVVMLETL SQSPPKGVTI PYRPKPSSSP VIFAGGQDRY
STGSDSASFP HTTPSMCLNP DLEGPPLEAY TIQGQYAIPQ PDLTKLHQLA
MQQSHFPMTH GNTGFSGIES SSPEVKGYWG LDASAQTTSH ELTIPNDLIG
CIIGRQGAKI NEIRQMSGAQ IKIANPVEGS TDRQVTITGS AASISLAQYL
INVRLSSETG GMGSS

Label-free MS quantitation using spectral counts

Protein	Accession number	Mutant	Wild-type
HuR (ELAV-like protein 1)	ELAV1_HUMAN	0	2
Poly(rC)-binding protein 2	PCBP2_HUMAN	7	10
Poly(rC)-binding protein 1	PCBP1_HUMAN	2	3

Figure 38. RNA:protein interactions enriched by the Thermo Scientific Pierce Magnetic RNA-Protein Pull-Down Kit can be detected via mass spectrometry. Proteins bound to the wild-type or mutant versions of the androgen 3'-UTR RNA sequence were enriched and analyzed by mass spectrometry. Samples enriched using the Pierce Magnetic RNA-Protein Pulldown Kit were prepared using the Thermo Scientific Pierce In-Gel Tryptic Digestion Kit. Each sample was diluted to 50 µL with 0.1% TFA and 5 µL was analyzed by LC-MS using a Thermo Scientific Velos Pro Mass Spectrometer with a top-20 data dependent method. Peptides were separated using a 5–40% gradient (buffer A: 0.1% FA; buffer B: 0.1% FA/100% acetonitrile) over 40 minutes using a ProteoPep II C18 15 cm column (New Objective) and ionized using a Thermo Scientific Nanospray Flex Ion Source instrument. MS spectra were searched using Mascot software against a mammalian SwisProt database using carbamidomethyl as a fixed cysteine modification and methionine oxidation as a variable modification. Scaffold Q+ software was used to analyze the search results using 99% peptide confidence and 90% protein confidence levels with a minimum of two peptides identified per protein. Protein sequence and peptide coverage are indicated in yellow. The spectral counts reveal that RNA mutation of the HuR binding site reduced HuR binding, while poly(rC)-binding protein1 and protein2 showed no significant decrease in binding.

Ordering information

Description	Quantity	Cat. No
Pierce RNA 3' End Desthiobiotinylation Kit Sufficient For: 20 desthiobiotinylation reactions using 5 pmol of RNA each Kit Contents: Desthiobiotinylated Cytidine Bisphosphate, 40 µL T4 RNA Ligase, 40 µL T4 RNA Ligase Reaction buffer (10X), 100 µL Non-labeled RNA Control, 100 µL Biotinylated IRE RNA Control, 35 µL RNase Inhibitor, 2 x 10 µL DMSO, 200 µL PEG (30%), 300 µL Glycogen, 20 µL Nuclease-free Water, 1.5 mL	20-rxn kit	20163
Pierce Magnetic RNA-Protein Pull-Down Kit Sufficient For: 20 RNA-protein complex pull-down reactions Kit Contents: RBP Enrichment Module (Cat. No 20164Y, store at 4°C): Pierce Nucleic Acid-Compatible Streptavidin Magnetic Beads, 1 mL RNA Capture Buffer (1X), 10 mL Tris (20mM, pH 7.5), 5 mL Protein-RNA Binding Buffer (10X), 1 mL Wash Buffer (1X), 10 mL Biotin Elution Buffer, 1.5 mL HuR Monoclonal Antibody (Mouse), 50 µL RNA Controls (Cat. No 20164Z, store at –20°C): Positive RNA Control (AR RNA), 250 pmol Negative RNA Control (polyA25 RNA), 250 pmol Pierce RNA 3' End Desthiobiotinylation Kit (Cat No. 20163, store at –20°C) (shipped separately on dry ice)	20-rxn kit	20164

Magnetic biotin pull-down

Perform high-capacity protein purification

Thermo Scientific™ Pierce™ Streptavidin Magnetic Beads provide easy affinity purification of biotin-labeled target molecules without columns or centrifugation. Pierce Streptavidin Magnetic Beads use a recombinant form of streptavidin with a mass of 53 kDa and a near-neutral isoelectric point (pI). The protein is a tetramer having four biotin-binding sites. Unlike avidin, streptavidin has no carbohydrate groups, resulting in low nonspecific binding. The high-affinity interaction between streptavidin and biotin cannot be dissociated efficiently except under very harsh conditions, such as boiling in sample loading buffer for SDS-PAGE or 8 M guanidine•HCl, pH 1.5. Consequently, it is often possible to elute binding partners in an interaction complex without also eluting the biotinylated component.

Highlights

- **Stable immobilization chemistry**—streptavidin is immobilized using leach-resistant chemistry
- **High capacity**—excellent-quality beads with high binding capacity provide rapid and efficient biomolecule purification from complex samples
- **Low nonspecific binding**—stable, pre-blocked beads provide clean purification products that are compatible with mass spectrometry analysis
- **Superior performance**—binding capacity is nearly three times higher than beads from other suppliers, allowing the use of smaller samples per experiment

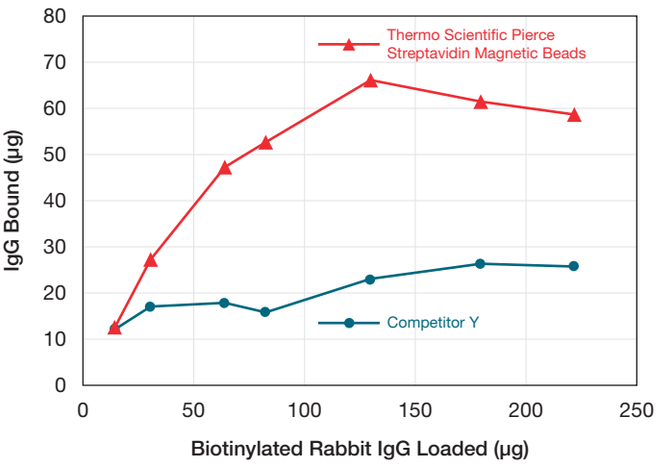


Figure 40. Higher binding capacity with Thermo Scientific Pierce Streptavidin Magnetic Beads. Pierce Streptavidin Magnetic Beads and competitor Y streptavidin magnetic beads were added to a 96 deep-well plate (1 mg beads per well). Using the KingFisher 96 Instrument, the beads were washed with phosphate-buffered saline containing 0.05% Tween-20. The beads were then incubated for one hour with varying amounts of biotinylated rabbit IgG (20–225 µg).

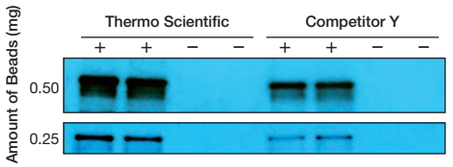


Figure 39. Better immunoprecipitation results with Thermo Scientific Pierce Streptavidin Magnetic Beads. MOPC cell lysate (0.75 mg per sample) was incubated overnight at 4°C with and without 10 µg biotinylated Grp94 antibody. Pierce Streptavidin Magnetic Beads and competitor Y streptavidin magnetic beads were added to a 96 deep-well plate (0.5 mg or 0.25 mg per well). Eluates were resolved by SDS-PAGE and analyzed by Western blot with anti-Grp94 antibody. About 0.25 mg of Pierce Streptavidin Magnetic Beads gave the same yield as 0.5 mg of competitor Y streptavidin magnetic beads.

Ordering information

Description	Quantity*	Cat. No
Pierce Streptavidin Magnetic Beads Sufficient for: Binding approx. 55 µg biotinylated rabbit IgG per mg of beads (approx. 3500 pmol biotinylated fluorescein per mg of beads)	1 mL	88816
Pierce Streptavidin Magnetic Beads Sufficient for: Binding approx. 55 µg biotinylated rabbit IgG per mg of beads (approx. 3500 pmol biotinylated fluorescein per mg of beads)	5 mL	88817

* Available in bulk quantities

Magnetic DYKDDDDK-tagged protein purification

Perform high-capacity protein purification

Thermo Scientific™ Pierce™ Anti-DYKDDDDK Magnetic Agarose provides a fast, convenient method for purification and immunoprecipitation (IP) of DYKDDDDK-tagged proteins from in vitro protein expression systems, bacteria, yeast, and mammalian cells. The amino acid sequence DYKDDDDK, commonly known as 'FLAG', is recognized by a high-affinity rat monoclonal antibody (clone L5) that is covalently attached to a magnetite-embedded agarose core particle.

For protein purification, the magnetic agarose is added to a sample containing DYKDDDDK-tagged proteins with the tag on either the N- or the C-terminus. Captured proteins are then magnetically separated from the supernatant, and non-specifically bound proteins can be washed away before dissociating bound DYKDDDDK-tagged proteins with elution buffer. The magnetic agarose is removed from the solution using a magnetic stand or an instrument such as the KingFisher Flex Magnetic Particle Processor. Automated instruments are especially useful for higher throughput purifications and screening of purification conditions.

Highlights

- **Specific**—unique base beads and highly specific antibody minimizes off-target binding (low non-specific binding)
- **High purity**—optimized bind-wash-elute protocol enables high purity
- **High yield**—special antibody conjugation method enables high yield
- **Rapid**—entire purification protocol typically takes less than 40 minutes
- **Economical**—purification protocol allows multiple reuses
- **Versatile**—beads are compatible with manual and automated workflows (e.g., KingFisher instruments)

Product characteristics

Table 9. Characteristics of Pierce Anti-DYKDDDDK Magnetic Agarose.

Composition	anti-DYKDDDDK antibody covalently attached to a magnetic, highly crosslinked agarose support
Magnetization	ferrimagnetic with low remanence
Bead size	10–40 µm
Bead concentration	25% slurry in phosphate buffered saline, 0.01% Tween-20 detergent, 0.02% sodium azide, pH 7.2
Binding capacity	≥3.2 mg DYKDDDDK-tGFP-His protein (~32 kDa)/mL settled beads

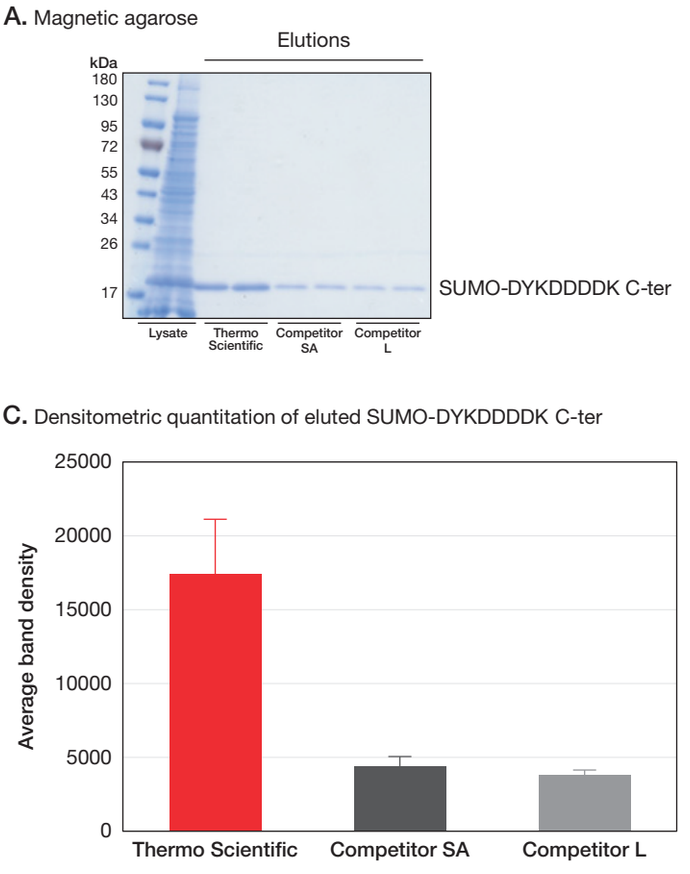


Figure 41. Higher yield of DYKDDDK- tagged SUMO protein. C- and N-terminal DYKDDDDK-tagged SUMO protein were expressed in E. coli and purified using Anti-DYKDDDDK Magnetic Agarose (A), respectively. Tagged protein was competitively eluted with 3X DYKDDDDK peptide and analyzed by SDS-PAGE and the iBright Imager (C). Comparison of the starting lysate and elutions show effective capture and elution of DYKDDDDK-tagged protein.

Magnetic DYKDDDDK-tagged protein purification, cont.

Perform high-capacity protein purification

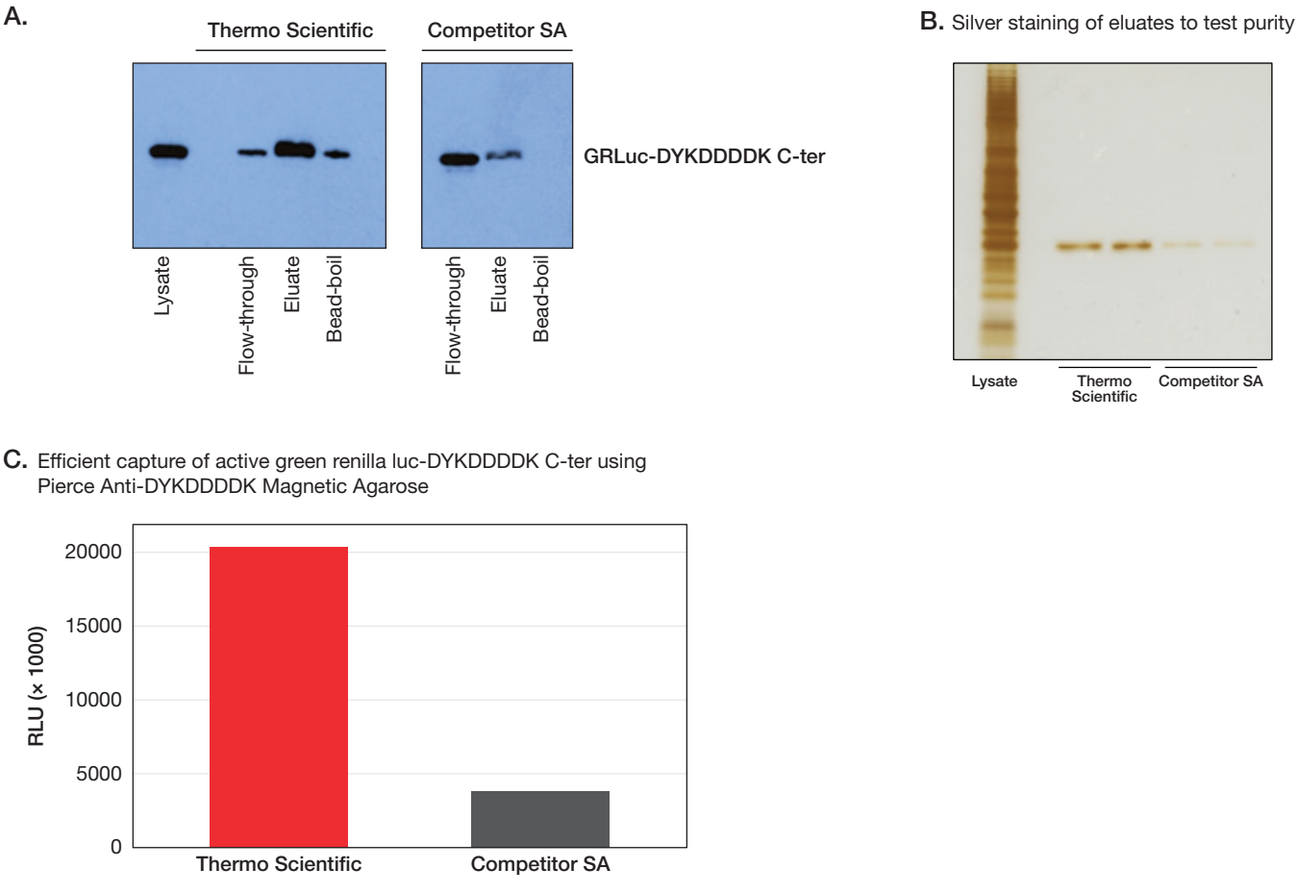


Figure 42. Efficient purification of DYKDDDDK-tagged green renilla luciferase. C-terminal DYKDDDDK-tagged green renilla luciferase protein was expressed in a HeLa IVT system (Cat. No. 88892) and immunoprecipitated using Pierce Anti-DYKDDDDK Magnetic Agarose or competitor SA anti-DYDDDDK magnetic beads using the KingFisher Flex Purification System. Tagged proteins were competitively eluted with 3X DYKDDDDK peptide and analyzed by western blot (A), silver stain (B), and Pierce Renilla Luciferase Glow Assay (C). Comparison of the starting lysate, elutions, and bead boiled samples show effective capture and elution of DYKDDDDK-tagged proteins with no background. Correlation of protein and activity levels indicate that a high level of green renilla luciferase activity is maintained after purification and competitive peptide elution.

Ordering information

Description	Quantity	Cat. No
Pierce Anti-DYKDDDDK Magnetic Agarose Sufficient for: ≥3.2 mg DYKDDDDK-tGFP-His protein (~32 kDa)/mL settled beads	1 mL	A36797
Pierce Anti-DYKDDDDK Magnetic Agarose Sufficient for: ≥3.2 mg DYKDDDDK-tGFP-His protein (~32 kDa)/mL settled beads	5 mL	A36798

Magnetic GST-tagged protein purification

Perform high-capacity protein purification

Thermo Scientific™ Pierce™ Glutathione Magnetic Agarose Beads provide a fast, convenient method for purification of glutathione-S-transferase (GST) from a bacterial, yeast, or mammalian crude cell lysate. Pierce™ Glutathione Magnetic Agarose Beads are well suited for purifying GST fusion proteins from a soluble protein extract and are optimal for the purification of proteins expressed at low levels from diluted supernatants. The beads can be used in manual applications with a magnetic stand or automated applications with an instrument such as the Thermo Scientific™ KingFisher™ Flex Magnetic Particle Processor. Automated instruments are especially useful for higher throughput purification and screening of purification conditions.

Highlights

- **High-performance beads**—non-aggregating, magnetite (Fe_3O_4), superparamagnetic beads provide exceptional uniformity for both manual and automated HTS applications
- **Stable affinity ligand**—glutathione is covalently immobilized to particles, enabling leach-resistance and clean purification products
- **High capacity**—binding capacity is sufficient for both routine and demanding purification procedures

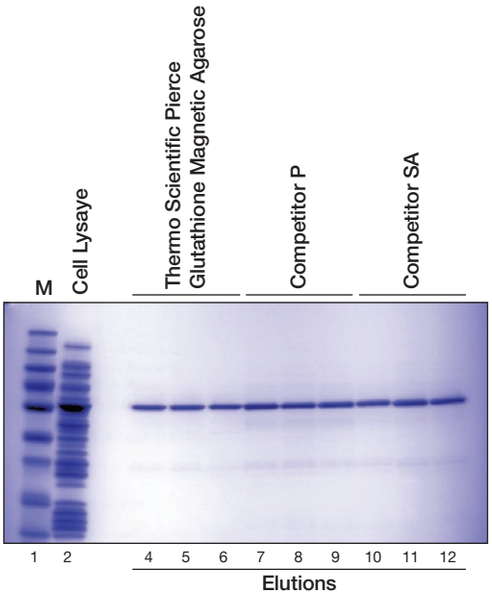
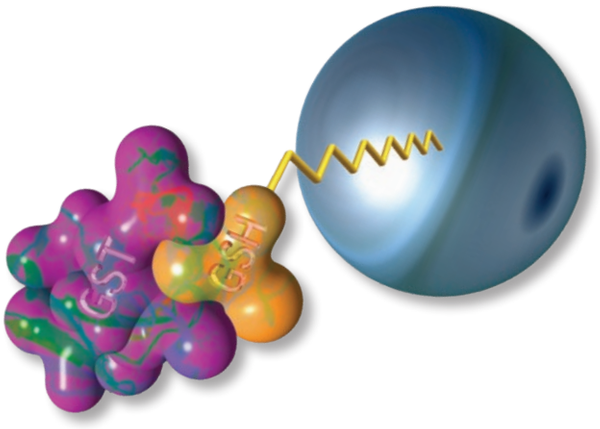


Figure 43. Pierce Glutathione Magnetic Agarose provides high purity purification of recombinant GST-tagged proteins. Cell lysates (250 µL) containing GST-tagged Green Renialla Luciferase were diluted with an equal volume of binding buffer and purified using magnetic agarose beads (25 µL settled volume) following manufacturer's protocol. Proteins were purified according to the manufacturer's instructions. Eluted fractions (2 µg total protein) were separated by SDS-PAGE and analyzed for percent purity. Lane 1: PageRuler Plus Prestained Protein Ladder, 10 to 250 kDa (Cat No. 26619), Lane 2: cell lysate, Lane 4–12: 2 µg of eluted fractions.



Ordering information

Description	Quantity*	Cat. No
Pierce Glutathione Magnetic Agarose Beads Sufficient for: Binding 5 to 10 mg GST per mL of beads	1 mL	78601
Pierce Glutathione Magnetic Agarose Beads Sufficient for: Binding 5 to 10 mg GST per mL of beads	5 mL	78602

* Available in bulk quantities

Magnetic His-tagged protein purification

Perform high-capacity protein purification

Thermo Scientific™ HisPur™ Ni-NTA Magnetic Beads are high-capacity Nickel-IMAC beads for affinity purification of His-tagged fusion proteins in manual or automated formats. The blocked magnetic bead surface is derivatized with the nitrilotriacetic acid (NTA) chelation moiety and loaded with divalent nickel ions (Ni^{2+}). The immobilized metal affinity chromatography (IMAC) beads provide high binding capacity with very low background. The HisPur Ni-NTA Magnetic Beads can be used both manually with a magnetic stand as well as with automated platforms such as the KingFisher Instruments for high-throughput needs.

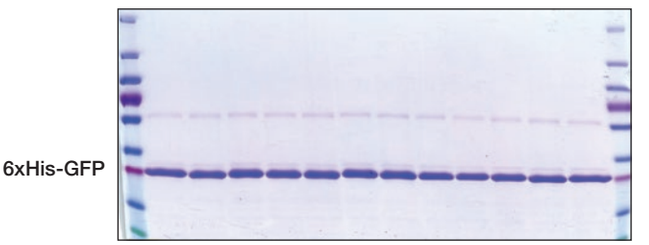
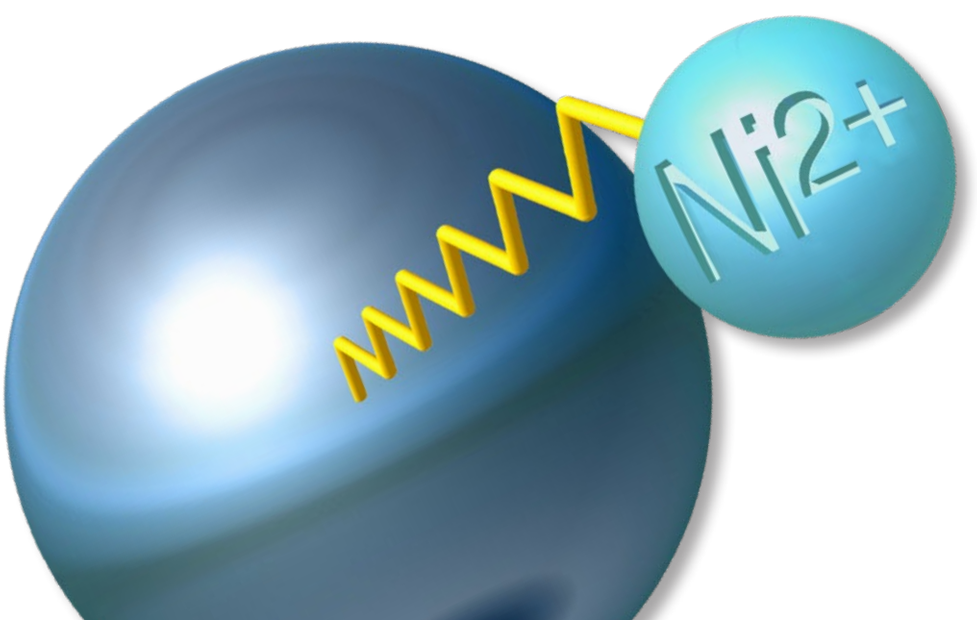


Figure 44. Thermo Scientific HisPur Ni-NTA Magnetic resin delivers consistent yield. His-tag protein purification was performed in a 96-well plate using a KingFisher Flex Instrument. In each well, 100 µg of *E. coli* lysate expressing 6xHis-GFP protein was added to 0.5 mg of Thermo Scientific Pierce Magnetic Ni-NTA Resin. Eluted protein was analyzed by SDS-PAGE stained with Imperial Protein Stain to determine well-to-well consistency in protein recovery. The variance between samples is measured at less than 15%.

Highlights

- **High capacity**—equivalent or higher binding capacity than Ni-NTA magnetic beads from other suppliers
- **Low nonspecific binding**—the bead surface is pre-blocked and the protocol provides optimized buffers for purification
- **Fast**—protocol is completed in less than one hour
- **Scalable**—process microliter to milliliter sample volumes
- **Versatile**—purify proteins using native or denaturing conditions
- **Reagent compatible**—can be used with common cell lysis reagents and a variety of buffer additives
- **Multiple formats**—protein coupling to the beads and downstream applications can be performed both manually and on an automated platform (e.g., KingFisher Instruments)



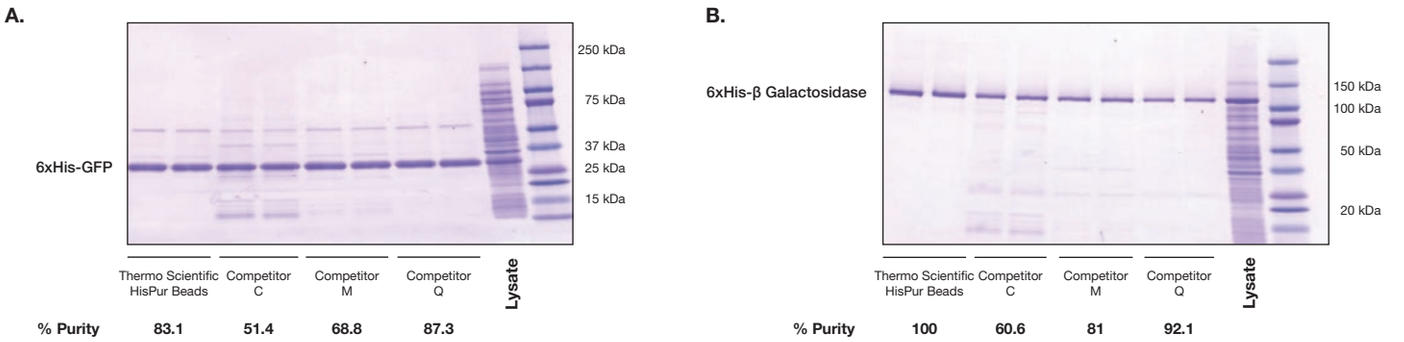


Figure 45. Excellent performance of Thermo Scientific HisPur Ni-NTA Magnetic Beads. Bacterial lysate (100 µL total protein) containing over-expressed 6xHis-GFP (Panel A) or over-expressed 6xHis-β Galactosidase (Panel B) was applied to 0.5 mg of HisPur Ni-NTA Magnetic Beads, crosslinked, beaded-form of agarose NiNTA magnetic beads (competitor C) NiNTA magnetic beads (competitor M) or NiNTA Magnetic Agarose Beads (competitor Q). All samples were run in duplicate, and the beads were processed using buffers recommended by the manufacturers. For the HisPur Ni-NTA Magnetic Beads, the amount of imidazole in the equilibration, wash and elution buffers was 30 mM, 50 mM and 150 mM, respectively. All three buffers contained 100 mM sodium phosphate and 600 mM sodium chloride. Binding was performed with all samples for 30 minutes. The beads were collected on a magnetic stand and the flow-throughs were saved for analysis. Eluates were resolved on an SDS-PAGE gel and stained with Imperial Stain. For purification of 6xHis-GFP, comparable yields and purity were observed for HisPur Ni-NTA and competitor Q Ni-NTA magnetic beads. HisPur Ni-NTA Magnetic Beads showed higher yield and purity than competitor Q Magnetic-Agarose Ni-NTA beads in the purification of 6xHis-β Galactosidase. The crosslinked, beaded-form of agarose Ni-NTA magnetic beads (competitor C) and Ni-NTA magnetic beads from competitor M gave lower purity and lower yield than HisPur Ni-NTA Magnetic Beads in both purifications. Purity analyses were performed on a Thermo Scientific MYECL Imager with Thermo Scientific MYImageAnalysis Software. Purity was determined by measuring the ratio of the background-corrected 6xHis-tagged protein band of interest to the sum of all bands in a given lane.

Table 10. Characteristics of Thermo Scientific HisPur Ni-NTA Magnetic Beads.

Composition	Ni^{2+} loaded on nitrilotriacetic acid that has been covalently coupled to the beads
Mean Diameter	1 µm (nominal)
Density	2.0 g/cm^3
Bead Concentration	12.5 mg/mL in 20% ethanol
Binding Capacity	≥40 µg of 6X-His-tagged GFP/mg of beads; ≥500 µg of 6X-His-tagged GFP/mL of beads

Ordering information

Description	Quantity*	Cat. No
HisPur Ni-NTA Magnetic Beads	2 mL	88831
HisPur Ni-NTA Magnetic Beads	10 mL	88832

* Available in bulk quantities

Magnetic His-tagged protein purification, cont.

Perform high-capacity protein purification

Thermo Scientific™ Pierce™ Ni-NTA Magnetic Agarose Beads provide a fast, convenient method for purification of His-tagged recombinant proteins. The beads are incubated with cell lysate containing His-tagged protein and then magnetically separated from the supernatant manually or through automation using an instrument such as the Thermo Scientific™ KingFisher™ Flex Magnetic Particle Processor. Nonspecifically bound protein can be washed away before dissociating bound His-tagged protein with elution buffer. Automated instruments are especially useful for higher throughput purification and screening of purification conditions.

Pierce Ni-NTA Magnetic Agarose Beads consist of highly crosslinked agarose beads embedded with magnetite and a covalently attached tetradentate nitrilotriacetic acid (NTA) chelator charged with divalent nickel ions. The density of the ligand on the magnetic agarose bead results in a binding capacity similar to or better than traditional agarose resins with the added feature of magnetic handling. Magnetic agarose beads are a valuable tool for small-scale (~1 mg) purification of multiple His-tagged proteins and for scouting expression and purification conditions to be used in larger-scale purifications with agarose chromatography supports.

Highlights

- **High capacity**—sufficient for both routine and demanding purification procedures; binds >70 mg of 6xHis-tagged protein per mL of settled resin
- **High-performance and reproducible beads**—non-aggregating, magnetite (Fe_3O_4), superparamagnetic beads provide exceptional uniformity for both manual and automated HTS purification applications
- **Low non-specific binding**—optimized purification protocol results in better tagged-protein purification
- **Versatile**—purify proteins using native or denaturing conditions
- **Compatible**—use with Thermo Scientific Cell Lysis reagents and a variety of buffer additives

The high-performance, magnetite-containing, superparamagnetic magnetic agarose resin is validated and optimized for use with high-throughput magnetic platforms, such as the KingFisher 96 and KingFisher Flex magnetic particle processors, but the beads also enable premium performance for simple benchtop purification applications using an appropriate magnetic stand.

Ni-NTA Magnetic Agarose Beads are used for small-scale affinity purification as well as high-throughput screening of recombinant His-tagged proteins. The polyhistidine tag is the most popular affinity tag and typically consists of six consecutive histidine residues (6xHis). Tagged proteins are overexpressed in a number of different systems, most commonly in bacteria, and purified from cell lysates such as those prepared using Thermo Scientific B-PER Bacterial Protein Extraction reagents. Purification of His-tagged proteins is achieved using an NTA chelate charged with nickel that coordinates with the histidine side chains. The NTA chelate contains four metal-binding sites that allow for low metal ion leaching and high binding capacity. The protocol for the Ni-NTA Magnetic Agarose Beads has been optimized to allow for high purity of the isolated His-tagged protein.

Product characteristics

Table 11. Characteristics of Pierce Ni-NTA Magnetic Agarose.

Composition	Magnetite-embedded 6% agarose coupled to an NTA-chelating ligand loaded with nickel ions
Magnetization	ferrimagnetic with low remanence
Bead size	10–40 µm
Bead concentration	25% slurry in 20% ethanol
Binding capacity	>75mg His-tagged green fluorescent protein (GFP) per mL settled beads

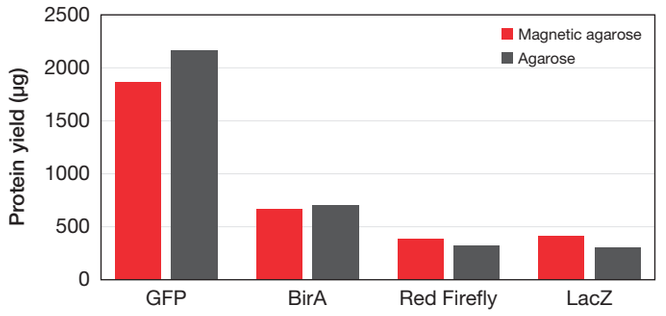


Figure 46. Magnetic vs. non-magnetic agarose purification. Thermo Scientific™ Pierce™ Ni-NTA Magnetic Agarose Beads provide purification yields in comparison to non-magnetic agarose. 6x His-tagged GFP, BirA, Red Firefly luciferase, and LacZ were purified from cell lysates using a manual application with both Ni-NTA Magnetic Agarose Beads and HisPur™ Ni-NTA Resin (Cat. No. 88221).

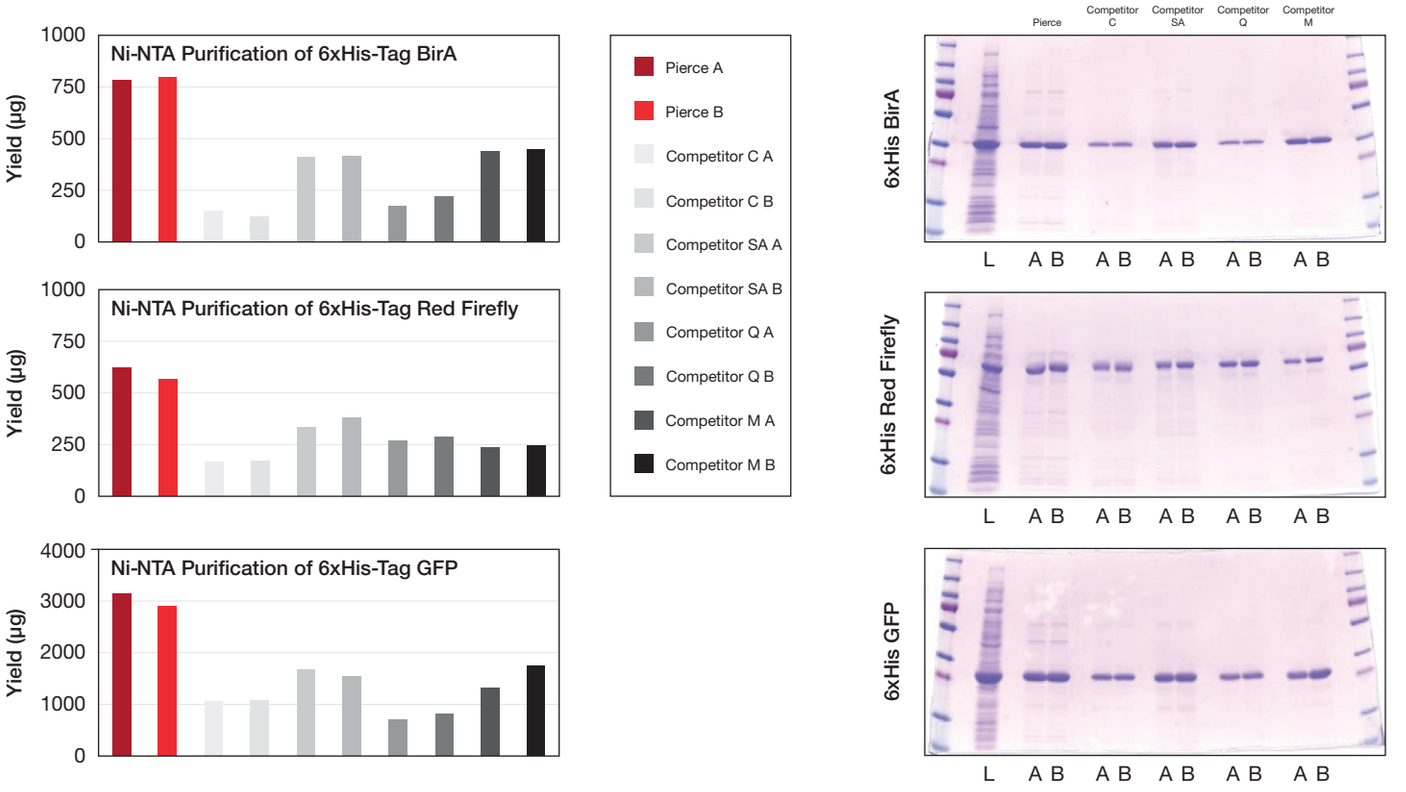


Figure 47. High purity His-tagged protein purification. 6xHis-Tagged lysates (250 mL) were diluted with binding buffer according to manufacturer's protocol and added to magnetic agarose beads . 6xHis-Tag proteins were purified following the manufacture's protocol. 6xHis-Tagged protein yields were estimated by Pierce Detergent Compatible Bradford at an absorbance of 595 nm. All purifications were done in duplicate (noted A and B).

Ordering information

Description	Quantity	Cat. No
Pierce Ni-NTA Magnetic Agarose Beads Sufficient for: >75mg His-tagged green fluorescent protein (GFP)/mL settled beads	1 mL	78605
Pierce Ni-NTA Magnetic Agarose Beads Sufficient for: ≥3.2 mg DYKDDDDK-tGFP-His protein (~32 kDa)/mL settled beads	5 mL	78606

Magnetic His-tagged protein purification, cont.

Perform high-capacity protein purification

Pierce high-capacity EDTA-compatible Ni-IMAC MagBeads are designed for purification of over-expressed His-tagged proteins from cell culture media. The beads have high binding capacity (up to 80 mg of protein per mL of settled beads) and can tolerate the high EDTA and reducing agent concentrations that are used to inhibit metalloproteases and stabilize sensitive intracellular proteins.

Highlights

- **Chelator stable**—stable in buffer containing 20 mM EDTA and DTT
- **Compatible**—overexpressed, secreted proteins in cell culture media (e.g., Expi293, ExpiCHO, and ExpiSf expression systems) can easily be purified
- **Exceptional high protein binding capacity**—up to 80 mg/mL of settled beads
- **High-performance**—non-aggregating, superparamagnetic microparticles provide exceptional performance with manual and automated HTS applications (e.g., Thermo Scientific KingFisher instruments)

Pierce high-capacity EDTA-compatible Ni-IMAC MagBeads consist of crosslinked agarose beads embedded with magnetite. The density of the ligand on the magnetic agarose bead results in a binding capacity similar to or better than traditional agarose resins with the added benefits of having magnetic properties and being EDTA compatible. The MagBeads are a valuable tool for small-scale purification of multiple His-tagged proteins and for scouting expression and purification conditions to be used in larger-scale purifications.

Pierce high-capacity EDTA-compatible Ni-IMAC MagBeads are optimized for use with high-throughput magnetic platforms, such as the KingFisher 96 and KingFisher Flex magnetic particle processors, but the beads also compatible with simple benchtop purification applications using an appropriate magnetic stand. Automated instruments, however, are especially useful for higher throughput purification and screening of purification conditions.

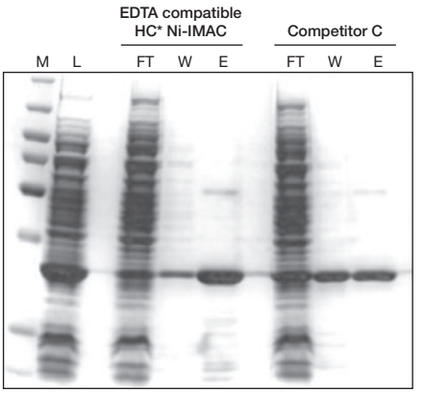


Figure 48. Superior performance compared to competitor. Bacterial lysate (100 mg total protein) containing over-expressed 6xHis-GFP was applied to Pierce High-Capacity Ni-IMAC MagBeads, EDTA compatible, as well as Competitor C magnetic beads. Beads were processed using protocols with buffers recommended by the manufacturers. Binding was performed with all samples for 30 minutes. The beads were collected on a magnetic stand and the flow-through (unbound) fractions were saved for analysis. The beads were then washed twice, and bound protein was eluted with a 15-minute incubation in Elution Buffer. The eluates were resolved on an SDS-PAGE gel and stained with Pierce Silver Stain Kit (Cat. No. 24612). Gel lanes were normalized to equivalent volume. M = MW marker, L = lysate load, FT = flow-through, W = wash, and E = elution.

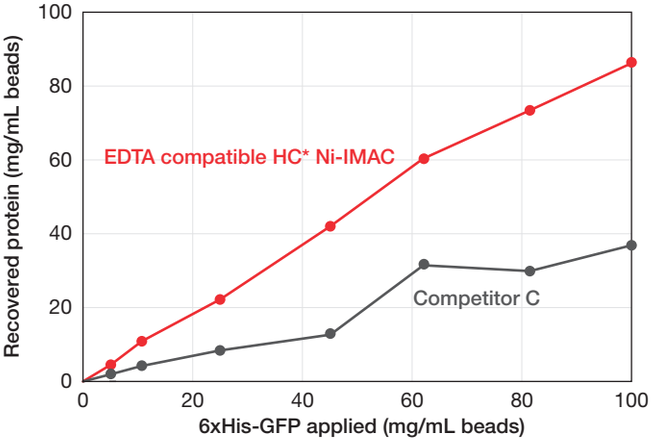


Figure 49. Superior capacity compared to competitor. Purified over-expressed 6xHis-GFP was applied to Pierce High-Capacity Ni-IMAC MagBeads, EDTA compatible, as well as Competitor C magnetic beads (0-100 mg GFP per 10 µL settled beads). Beads were processed using protocols with buffers recommended by the manufacturers on the Kingfisher Flex system. Binding was performed with all samples for 30 minutes. The beads were collected, and the flow-through (unbound) fractions were saved for analysis. The beads were then washed twice, and bound protein was eluted with a 15-minute incubation in Elution Buffer. *HC = High-Capacity

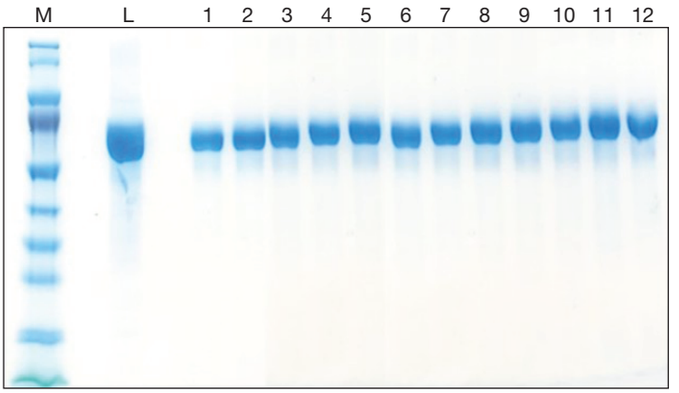


Figure 50. Consistency of purifications. Cell culture supernatant (Expi293) containing over-expressed His-tagged HSA (50 mg total protein) was applied to Pierce High-Capacity Ni-IMAC MagBeads, EDTA compatible (10 µL settled beads) in a 96-well plate. Beads were processed using the Kingfisher Flex system. Binding was performed with all samples for 30 minutes. The beads were collected, and the flow-through (unbound) fractions were saved for analysis. The beads were then washed twice, and bound protein was eluted with a 15-minute incubation in Elution Buffer. The eluates were resolved on an SDS-PAGE gel and stained with GelCode Blue (Cat. No. 24594). Gel lanes were normalized to equivalent volume. M = MW marker, L = lysate load, 1–12 = corresponding elution from plate row. CV's <10%.

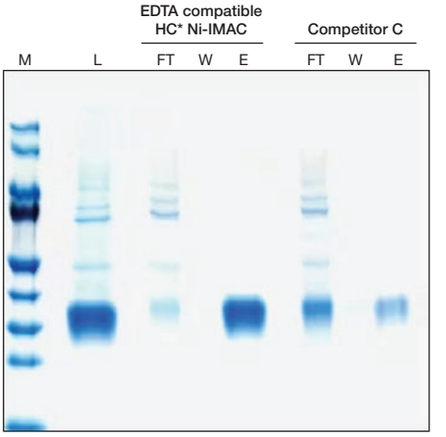
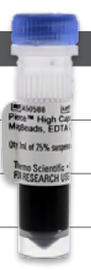


Figure 51. Superior performance compared to competitor. Cell culture supernatant (Expi293) containing over-expressed His-tagged EPO (40 mg total protein) was applied to Pierce High-Capacity Ni-IMAC MagBeads, EDTA compatible, as well as Competitor C magnetic beads. Beads were processed using protocols with buffers recommended by the manufacturers. Binding was performed with all samples for 30 minutes. The beads were collected on a magnetic stand and the flow-through (unbound) fractions were saved for analysis. The beads were then washed twice and bound protein was eluted with a 15-minute incubation in Elution Buffer. The eluates were resolved on an SDS-PAGE gel and stained with GelCode Blue (Cat. No. 24594). Gel lanes were normalized by volume. M = MW marker, L = lysate load, FT = flow-through, W = wash, and E = elution. *HC = High-Capacity

Ordering information

Description	Quantity	Cat. No
Pierce™ High Capacity Ni-IMAC MagBeads, EDTA compatible Sufficient for: up to 80 mg of 6 xHis-tagged protein/mL of settled magnetic beads	1 mL	A50588
Pierce™ High Capacity Ni-IMAC MagBeads, EDTA compatible Sufficient for: up to 80 mg of 6 xHis-tagged protein/mL of settled magnetic beads	5 mL	A50589
Pierce™ High Capacity Ni-IMAC MagBeads, EDTA compatible Sufficient for: up to 80 mg of 6 xHis-tagged protein/mL of settled magnetic beads	25 mL	A50590
Pierce™ High Capacity Ni-IMAC MagBeads, EDTA compatible Sufficient for: up to 80 mg of 6 xHis-tagged protein/mL of settled magnetic beads	100 mL	A50591



Magnetic MS Sample Prep Kits

Optimized, rapid protein extraction and digestion of samples for MS analysis

The newly expanded Thermo Scientific™ EasyPep™ MS Sample Prep product portfolio now includes magnetic kits for 20 samples or 96-well plate samples that enable efficient and reproducible processing of plasma, cultured mammalian cells, and tissues for MS analysis. These kits contain pre-formulated buffers, MS-grade enzyme mix, magnetic beads for peptide clean-up, and an optimized protocol to generate MS-ready peptide samples in less than 3 hours (Figure 52).

Highlights

- **Complete**—includes preformulated reagents for lysis through digestion, peptide cleanup columns, and an optimized protocol for processing up to 20 samples, or one 96-well plate
- **Optimized**—streamlined protocol and reagents minimize the number of steps and time it takes to process samples
- **Flexible**—reagents and protocol have been verified using cells, plasma, and tissue samples for 15 µg to 100 µg samples
- **Time-saving**—sample processing has been reduced from more than 1 day to less than 3 hours
- **Compatible**—sample is ready for MS analysis and other downstream applications, including label-free quantitation, phosphopeptide enrichment, and Thermo Scientific™ TMT™ and TMTpro™ reagent labeling

Magnetic methods are becoming more widely adopted for proteomics sample preparation due to the ability to automate protein clean-up. However, these methods face challenges related to sample and reagent compatibility, different magnetic bead options, and lack of standardized procedures. To address these challenges, we introduce a new EasyPep Magnetic sample preparation workflow utilizing a novel magnetic bead for streamlined and automated proteomics sample preparation. Compared to other magnetic resins, our resin exhibits excellent compatibility with a wider range of solvents (water, acetone, acetonitrile, methanol), pH (pH 3–12), and is free from leachables. We optimized the peptide binding and elution for different sample input amounts (1 µg–1.5 mg) and preparation of up to 96 samples using Invitrogen™ KingFisher™ system in under 3 hours. Validation studies using mammalian cells, plasma, and tissue samples (10–100 µg) demonstrated exceptional digestion efficiency with a missed cleavage rate of ~10% and complete cysteine reduction and alkylation. Notably, the eluted peptides can be directly injected into liquid chromatography-mass spectrometry (LC-MS), eliminating the conventional and time-consuming SpeedVac drying and reconstitution steps. Our sample preparation process exhibits high robustness and reproducibility with peptide and protein identifications having less than 5% CV and quantification of protein abundances showing less than 10% CV. To enhance the sample throughput of our automation solution, we integrated tandem mass tag (TMT™) for multiplexed proteome analysis.

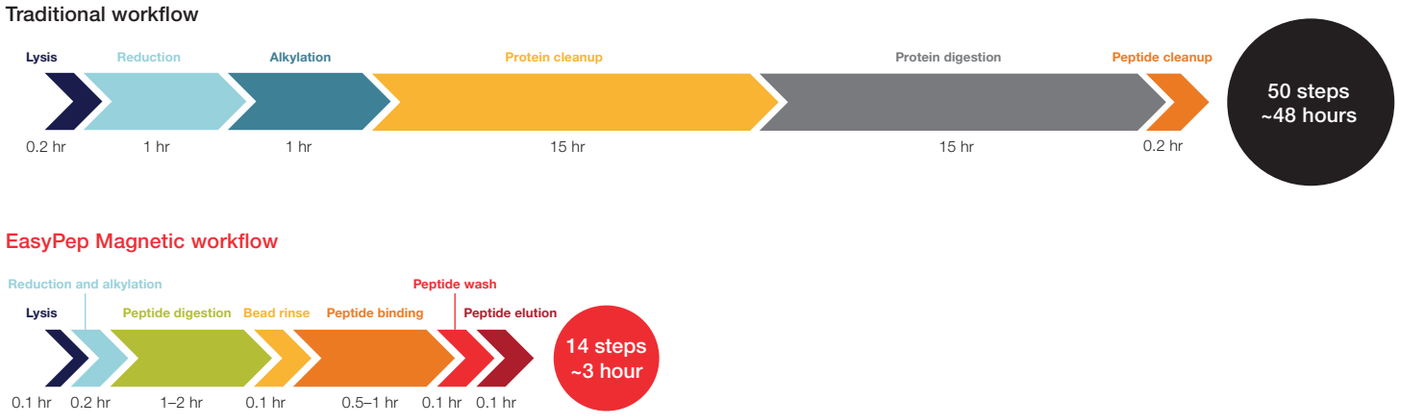


Figure 52. Comparison of sample preparation methods using a traditional workflow and a workflow using an EasyPep Magnetic MS kit. Compared to a traditional workflow, the EasyPep Magnetic MS Sample Prep Kits produce high-quality peptides in less time with half the number of manipulation steps.

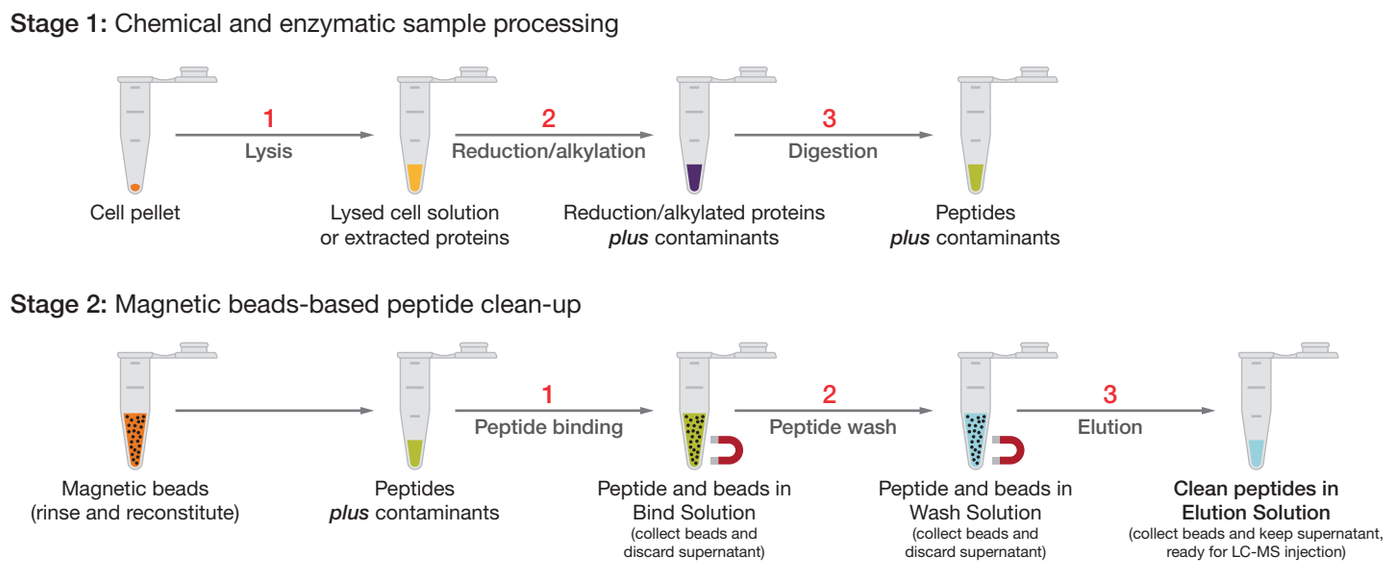


Figure 53. Schematic of EasyPep Magnetic proteomic sample preparation workflow. The workflow is optimized to process protein samples with a high yield of peptides that are MS-ready to inject without the need for SpeedVac within 3 hours. The EasyPep Magnetic MS Sample Prep Kit can process samples manually or through automation using an instrument such as the Thermo Scientific™ KingFisher™ Apex system.

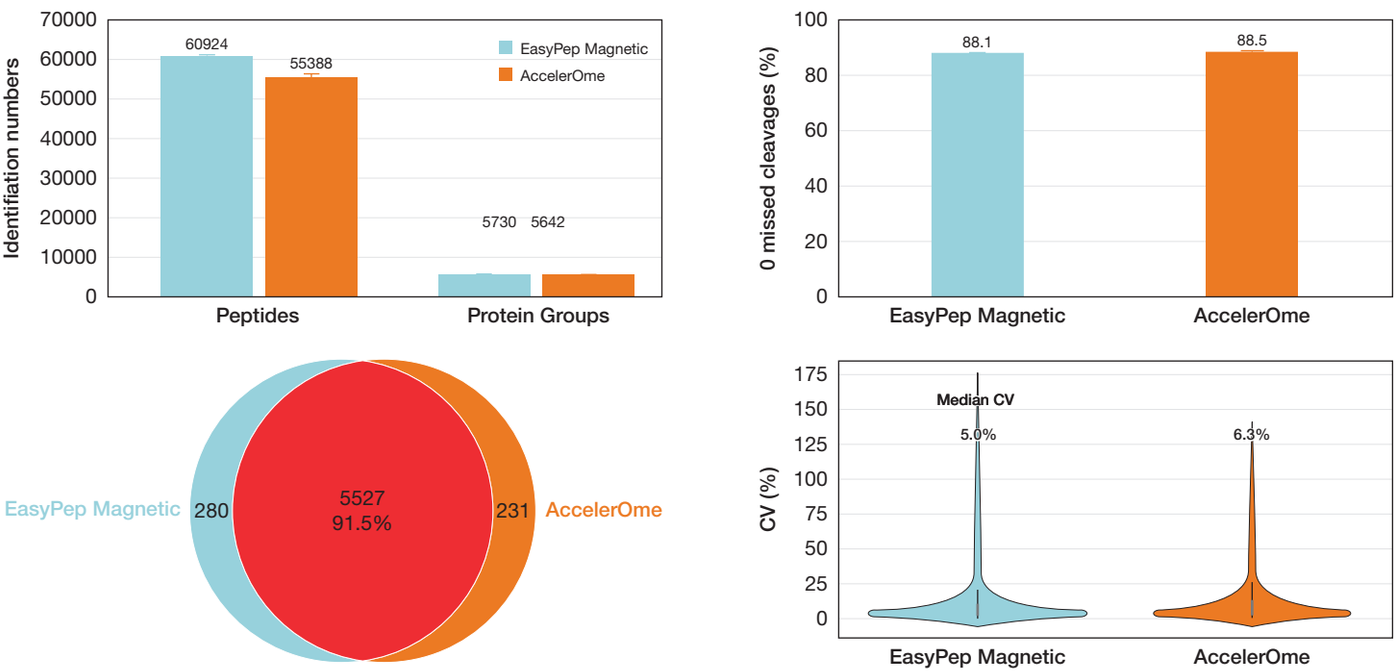


Figure 54. Benchmark of EasyPep Magnetic method against AccelerOme Automated method. (A) Comparable protein IDs; (B) High digestion efficiency (>88% zero peptide missed cleavages) using 1-hour digestion; (C) High overlaps of quantifiable proteins (>91%); (D) Good quantification reproducibility with median CV of protein abundances for both methods (<10%). In this experiment, digest from 25 µg of HeLa cell lysate was analyzed by Exploris 480 with DIA method.

Magnetic MS Sample Prep Kits, cont.

Optimized, rapid protein extraction and digestion of samples for MS analysis

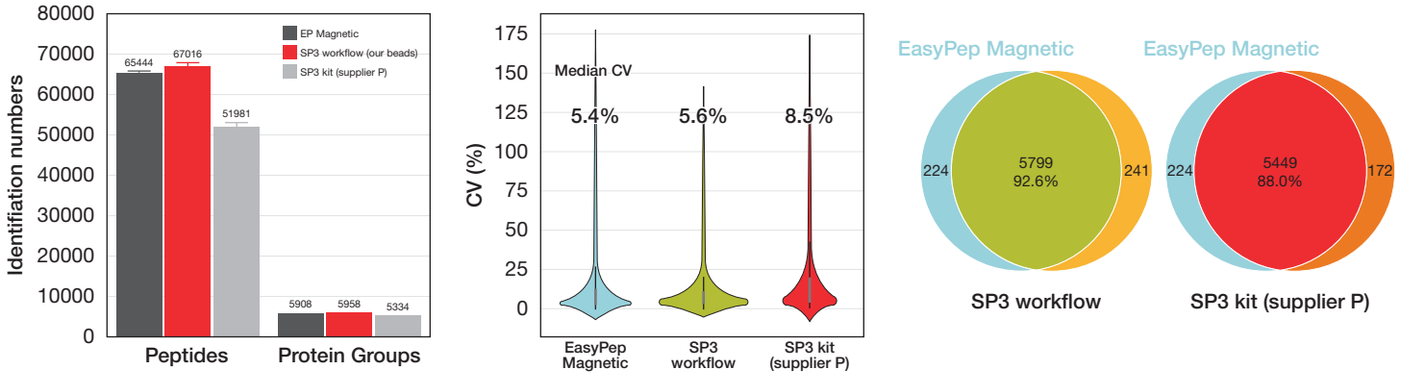


Figure 55. Benchmark of EasyPep Magnetic method against SP3 method using the same magnetic beads we developed and using an SP3 kit from a competitor. (A) The IDs were comparable between the EasyPep Magnetic method and the SP3 method using the same magnetic beads; >25% peptides and >10% proteins were observed compared to the SP3 kit method (supplier P). (B) The median CVs of protein abundances were <10% for all methods, indicating good quantification reproducibility. (C) The overlaps of quantifiable proteins between the EasyPep Magnetic and SP3 methods using the same magnetic beads and using the SP3 kit were ~93% and ~88%, respectively. In this experiment, digest from 50 µg of HeLa cell lysate was analyzed by Exploris 480 with DIA method. The digestion time was 1 hour for the EasyPep Magnetic method and 5 hours for the SP3 methods.

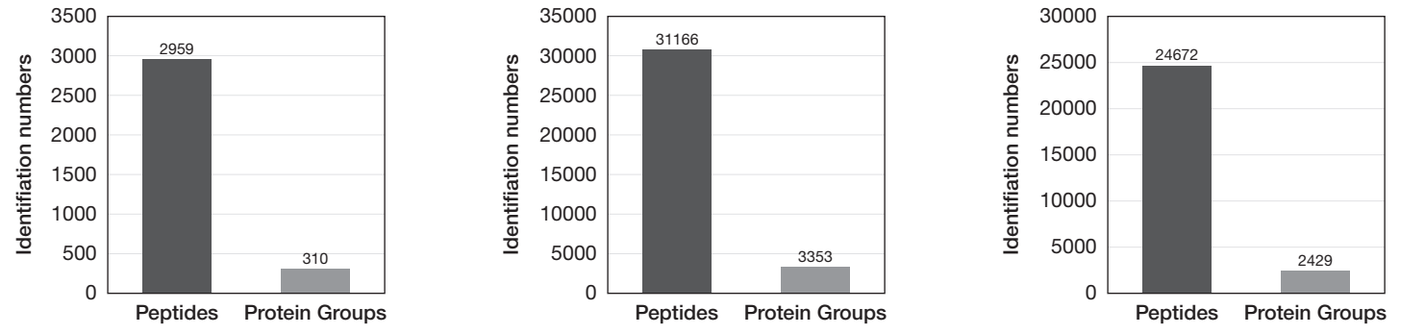


Figure 56. Performance of the EasyPep Magnetic for processing samples including (A) plasma, (B) tissue, and (C) bacteria. Reproducible peptide and protein identifications could be achieved for co mplex protein samples. The input amount was 25 µg per sample and digest was analyzed by Exploris 480 with DIA method.

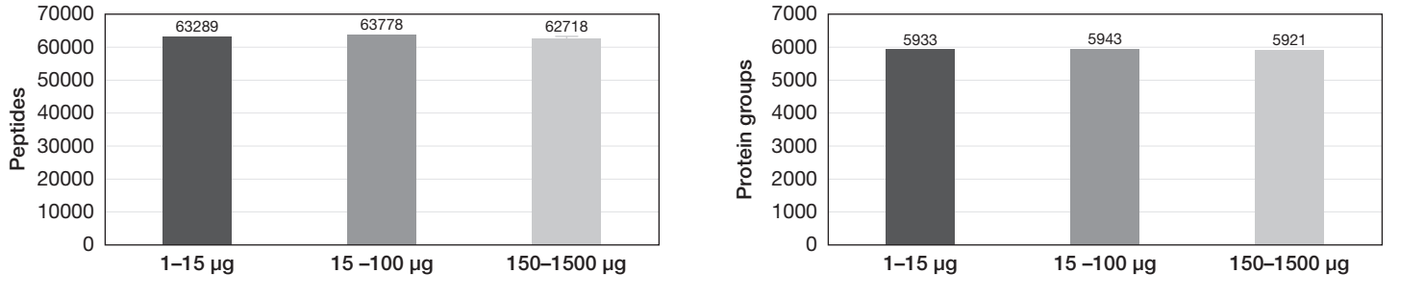


Figure 57. Performance of sample preparation for microgram (1–15 µg) and milligram (0.15–1.5 mg) sample range. Consistent peptide (A) and protein (B) identification numbers were obtained across the whole sample prep range. HeLa cell lysate and Exploris 480 with DIA method were used in this experiment.

Magnetic MS Sample Prep Kits

Proteomics magnetic clean-up beads

Magnetic beads offer rapid and efficient processing of multiple samples simultaneously, saving time and resources. In addition, magnetic bead-based workflows are amenable to automation, reducing the risk of human error and ensuring consistent results for high-throughput proteomics sample preparation and analysis. However, some challenges exist which can complicate its adoption into the field. These challenges include the variety magnetic bead options, potential issues with reproducibility, and the need for standardized procedures. Herein, we developed our Proteomics Magnetic Clean-up Beads with a workflow to enable fast, efficient, and reproducible removal of a wide variety of detergents, salts and other MS-incompatible contaminants using a variety of commercially available lysis reagents (Table 12, Figure 58).

Pierce Proteomics Magnetic Clean-up Beads have been designed for compatibility with common solvents (e.g. acetonitrile, ethanol, isopropyl alcohol, and acetone) and wide range of pH (e.g. pH 2–13) to enable fast, efficient, and reproducible removal of a wide variety of detergents, salts, and other MS-incompatible contaminants from protein and peptide samples. The beads can be successfully used to clean up proteins derived from different sample types (e.g. purified proteins, cell lysates, tissue extracts, and biological fluids) and protein input amounts (e.g. 1 µg to 1 mg) using manual magnetic processing or automated workflows (e.g. Thermo Scientific™ KingFisher™ instruments).

Highlights

- **Efficient**—the beads offer highly effective purification and clean-up of proteins from complex mixtures, such as cell lysates or biological samples.
- **Versatile**—the beads are compatible with a wide range of sample types, including cell lysates, serum, plasma, and tissue extracts.
- **High binding capacity**—the beads have a high binding capacity for proteins, enabling efficient capture and recovery of target proteins from complex mixtures.
- **Low non-specific binding**—the beads exhibit low non-specific binding, leading to cleaner and more specific isolation of target proteins.
- **Scalable workflow**—the beads can be used for both small-scale and large-scale protein purification, making them suitable for various experimental needs.

Table 12. Example of lysis reagents compatible with Pierce Proteomics Magnetic Clean-up Beads.

Lysis reagent	Description
Thermo Scientific™ NE-PER™ Nuclear and Cytoplasmic Extraction Reagents	Obtain nuclear and cytoplasmic fractions of soluble proteins from the same sample
Thermo Scientific™ PierceM GPCR Extraction and Stabilization Reagent	Optimized to extract and stabilize GPCRs for downstream activity assays
Thermo Scientific™ Mem-PER™ Plus Membrane Protein Extraction Kit	Obtain membrane and cytoplasmic fractions from the same sample
Thermo Scientific™ T-PER™ Tissue Protein Extraction Reagent	Optimized for mild extraction of total protein from tissue samples
Thermo Scientific™ Pierce™ IP Lysis Buffer	Optimized for IP and pull-down assays; maintains protein complexes
Thermo Scientific™ EasyPep™ MS Sample Prep Kit lysis buffer	General lysis buffer optimized for MS sample prep

Magnetic MS Sample Prep Kits, cont.

Proteomics magnetic clean-up beads

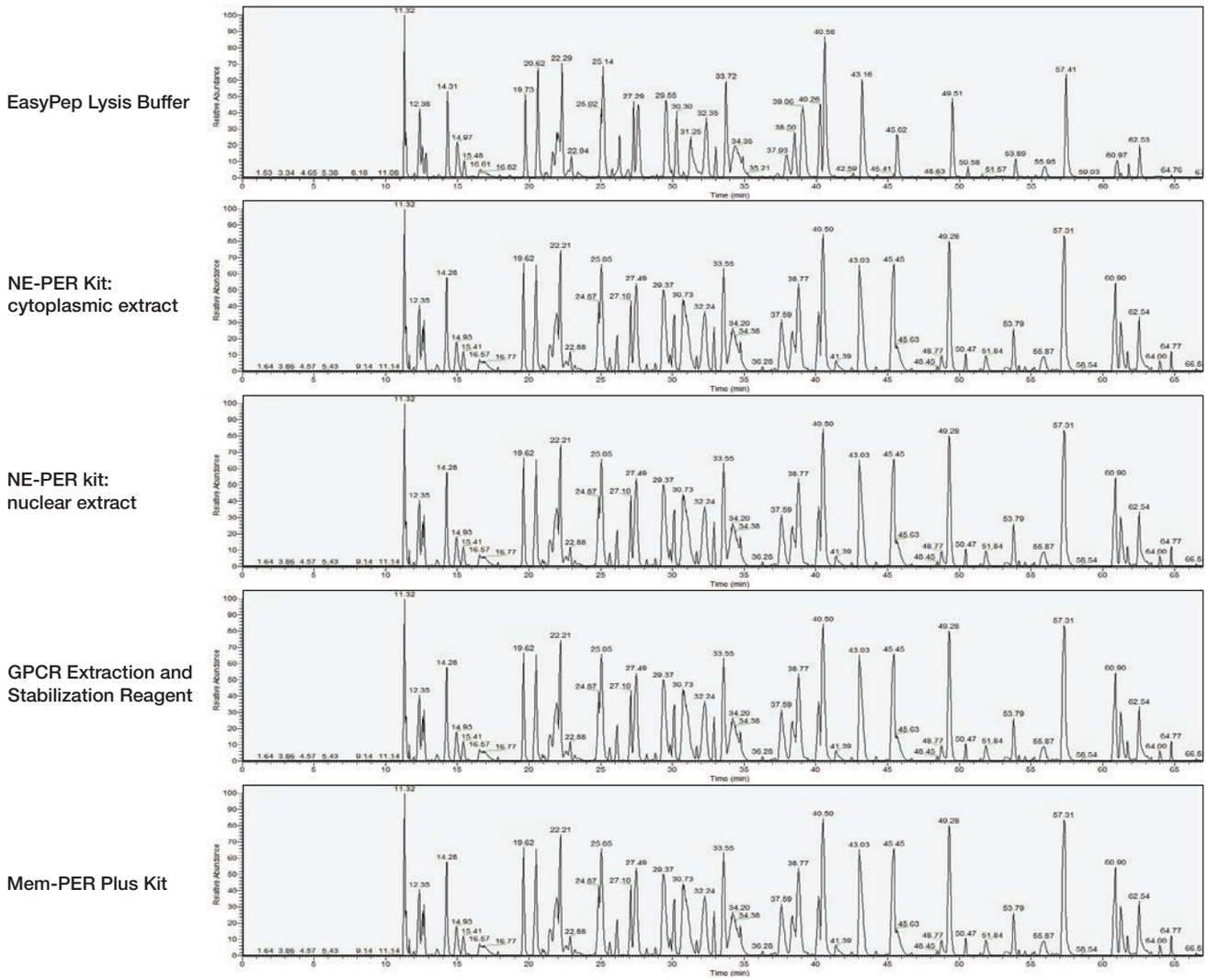


Figure 58. MS results of the Proteomics Magnetic Clean-up Bead workflow with BSA. The resulting BSA total ion chromatograms (TICs) show very similar peptides separation and peak resolutions, including the EasyPep Lysis Buffer that is referenced as a positive control. No visible detergent contaminant peaks are seen throughout the gradient indicates efficient removal of detergents using Proteomics Magnetic Clean-up Beads.

Table 13. BSA LC-MS/MS analysis. Sequence coverage, unique peptides, and 0% missed cleavages are shown for the 5 different BSA samples processed using the Proteomics Magnetic Clean-up Beads. >85% sequence coverage, >100 unique peptides, and >50% zero missed cleavages for BSA was obtained using all 5 different buffers. These LC-MS/MS results are very similar regardless of the starting lysis reagent.

	Sequence coverage	Unique peptides	% Zero missed cleavages
EasyPep lysis buffer	88%	102	58.6%
NE-PER kit, cytoplasmic extraction buffer	92%	114	63.6%
NE-PER kit, nuclear extraction buffer	86%	105	60.4%
GPCR Extraction and Stabilization Reagent	89%	100	57.8%
MemPER Plus, membrane extraction buffer	87%	102	52.9%

LC-MS/MS results of the Proteomics Magnetic Clean-up Bead workflow with different sample types and lysis reagents.

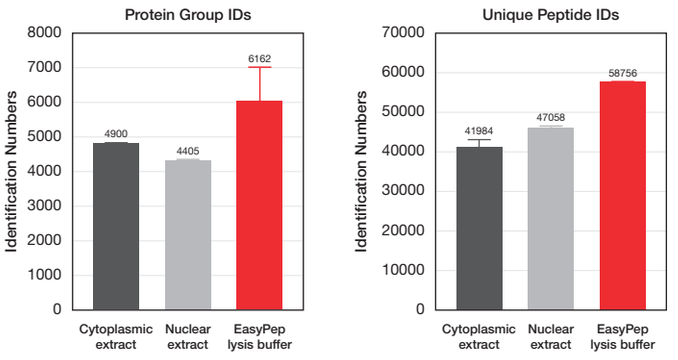


Figure 59. HEK293 cells were processed according to the manufacturer instructions, ending with two fractions from the same sample using the NE-PER kit. 50 µg of total protein was used for processing. Resulting protein group identifications (A) and unique peptide IDs (B) are shown.

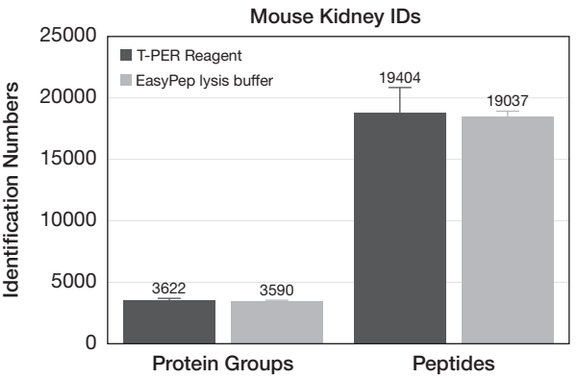


Figure 60. Frozen mouse kidney was processed according to the manufacturer instructions. 50 µg of total protein was used for processing with the Proteomics Magnetic Clean-up Beads workflow. Resulting protein group identifications and unique peptide IDs are shown.

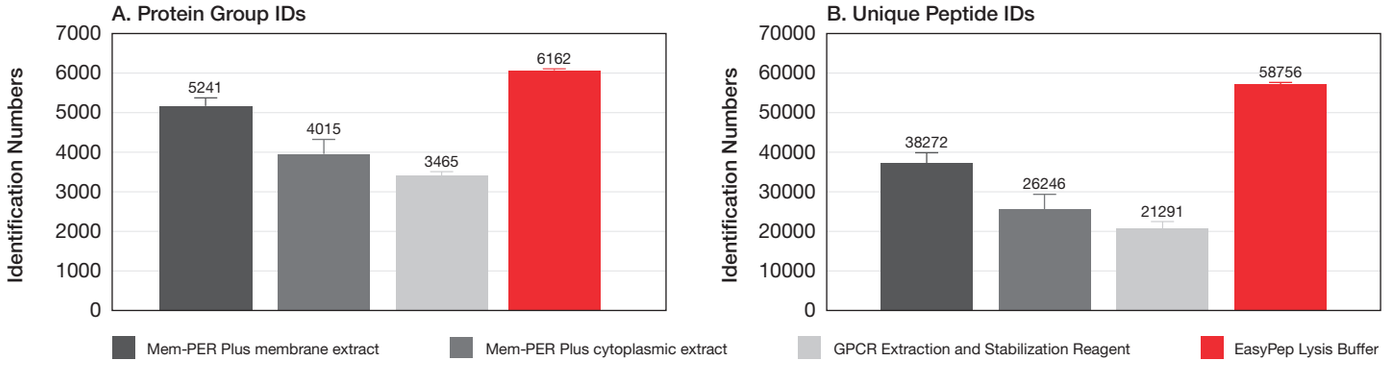


Figure 61. HEK293 cells were processed according to the manufacturer instructions for, ending with two fractions from the same sample using the Mem-PER Plus kit. 50 µg of total protein was used for processing. Resulting protein group identifications (A) and unique peptide IDs (B) are shown.

Ordering information

Description	Quantity	Cat. No
EasyPep™ Magnetic MS Sample Prep Kit	20 reactions	A57866
EasyPep™ Magnetic MS Sample Prep Kit	96 reactions	A57867
Pierce™ Proteomics Magnetic Clean-up Beads	5 mL	A57868

Find out more at thermofisher.com/easypep

Pierce MS-Compatible Magnetic IP Kit

Validated kits for the efficient and reproducible enrichment of target antigens for LC-MS analysis

The Thermo Scientific™ Pierce™ MS-Compatible Magnetic IP Kits provide MS-friendly reagents and optimized protocols to enable highly effective and efficient IP and co-IP of target antigens upstream of LC-MS analysis. In addition, low protein–binding microcentrifuge tubes are supplied separately to minimize loss during the sample processing.

Highlights:

- **MS–compatible**—directly compatible with in-solution peptide digestion
- **Flexible**—different IP strategies are available to utilize either native or biotinylated antibodies
- **Sensitive**—kits have been demonstrated to successfully enrich for low-abundance proteins
- **Low background**—buffers optimized to minimize enrichment of background proteins
- **Robust**—procedure and reagents have been robustly tested with numerous targets to enable consistent enrichment of low-abundance proteins

The Pierce MS-Compatible Magnetic IP Kits contain either high-quality Thermo Scientific™ Pierce™ Streptavidin or Protein A/G Magnetic Beads. The Pierce Protein A/G Magnetic Beads provide wider flexibility of antibody capture than using either Protein A or G alone.

The optimized components of each kit have been formulated to be compatible with downstream LC-MS analysis. After the immunoprecipitation procedure, the target-enriched elution fraction is ready for in-solution tryptic digestion, without the need for gel purification, detergent removal, or desalting. These kits have been rigorously validated using numerous target antigens with varying expression levels, including targets previously undetected without enrichment or by western blotting.

Additionally, the reagents and procedures have been validated using both manual and automated magnetic separation.

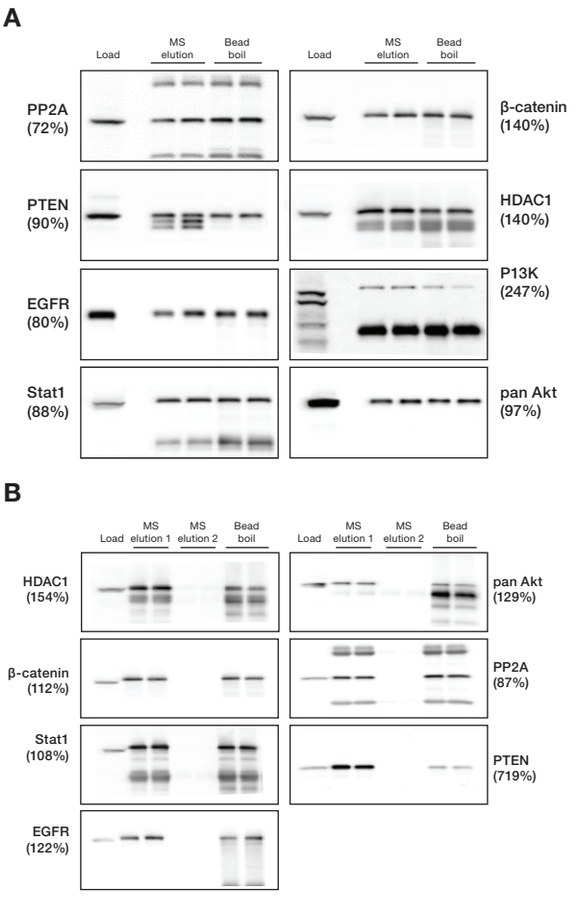


Figure 62. The Pierce MS-Compatible Magnetic Kits allow for effective target capture and elution. (A) Streptavidin kit. **(B)** Protein A/G kit. Percentages beneath target indicate elution efficiency compared to bead boil. The elutions were analyzed by western blot. Antibodies were labeled with the Pierce Antibody Biotinylation Kit for IP and used with the Pierce MS-Compatible Magnetic IP Kit (Streptavidin) to immunoprecipitate target proteins from cell lysates.

Table 14. Endogenous cellular targets identified with and without enrichment. The Pierce MS-Compatible Magnetic IP Kit (Streptavidin) was used to enrich 17 targets. Antibodies were labeled using the Pierce Antibody Biotinylation Kit for IP. Targets are generally grouped by their relative cellular abundance (high, medium, and low) and may be cell line dependent. Western blots were performed with the IP elutions. Western blot signal intensity is shown as low (+) to high (+++). MS analysis on unenriched lysates is indicated by "Yes" when at least two unique peptides were identified for the particular target. MS analysis (enriched) is denoted by "++" or indicating a medium or higher fold enrichment compared to the MS analysis on native lysate.

Target	Cellular abundance	Western blot (IP)	MS analysis (unenriched)	MS analysis (enriched)
PP2A	High	+++	Yes	++
HDAC1		+++	Yes	++
STAT1	Medium	+++	Yes	++
CBP		+	No	+++
PTEN	Low	+++	Yes	++
EGFR		++	Yes	+++
AKT2		++	Yes	+++
AKT1		+	No	+++
CTNNB1		++	No	+++
PI3K		+	No	+++
SMAD4		+	Yes	++
ERBB2		+	No	+++
TP53		+	No	+++
CDH2		–	No	+++
LKB1		ND	No	+++
NOTCH1		ND	No	+++
NOTCH2		ND	Yes	+++

Table 15. Endogenous cellular targets identified with and without enrichment. The Pierce MS-Compatible Magnetic IP Kit (Protein A/G) was used to enrich 20 targets. Targets are generally grouped by their relative cellular abundance (high, medium, and low) and may be cell line dependent. Western blots were performed with the IP elutions. Western blot signal intensity is shown as low (+) to high (+++). MS analysis on unenriched lysates is indicated by "Yes" when at least two unique peptides were identified for the particular target. MS analysis (enriched) is denoted by a "++" or indicating a "medium" or higher fold enrichment compared to the MS analysis on native lysate.

Target	Cellular abundance	Western blot (IP)	MS analysis (unenriched)	MS analysis (enriched)
PP2A	High	+++	Yes	++
HDAC1		+++	Yes	++
STAT1	Medium	+++	Yes	++
CBP		+	No	+++
PTEN	Low	+++	Yes	++
EGFR		++	Yes	+++
AKT2		++	Yes	+++
AKT1		+	No	+++
CTNNB1		++	No	+++
PI3K		+	No	+++
SMAD4		+	Yes	++
ERBB2		+	No	+++
KRAS		+	No	+++
TP53		ND	No	+++
CDH2		ND	No	+++
ARAF		ND	Yes	+++
BRAF		ND	No	+++
LOK		ND	No	+++
NOTCH1		ND	No	+++
NOTCH2		ND	Yes	+++

Pierce MS-Compatible Magnetic IP Kit

Validated kits for the efficient and reproducible enrichment of target antigens for LC-MS analysis

Table 16. List of co-immunoprecipitated proteins. The Pierce MS-Compatible Magnetic IP Kits showed effective co-IP of interacting proteins for CTNNB1, EGFR, PI3KCA, CBP, NOTCH1, AKT, AKT1, SMAD4, and/or ARAF targets. These are known protein interactions reported in previous studies. Panel A: Streptavidin Kit. Panel B: Protein A/G Kit.

Panel A

IP target	Co-IP proteins
CTNNB1	CTNNA1, CDH2, CDH11, APC, ARVCF, PKP4
EGFR	PRKDC, PFKP, SL C3A2, RPN1
PI3KCA	PIK3R2, PIK3R1
CBP	PSMC5, ACTA2, DDX5
AKT	VIM, HSPA8, TUBA1A
SMAD4	EEF1A1, SQSTM1

Panel B

IP target	Co-IP proteins
CTNNB1	CTNNA1, CDH11, CDH2, CTNND1
EGFR	TUBB, TUBA1A, HSPA1A
PI3KCA	PIK3R2, PIK3R1
NOTCH1	PTBP1, C14orf166
AKT1	AKT2, ACTB
ARAF	YWHAG, STK25

Table 17. IP-MS product selection guide.

	Protein A/G IP-MS kit	Streptavidin IP-MS kit
		
Surface coating on bead	Protein A/G	Streptavidin
Type of ligand required	Primary antibodies from most species	Any biotinylated antibody or ligand
IP protocol time	2–3 hr	2–3 hr
Main benefits	• Easiest protocol • Binds most antibodies • High yield, low nonspecific binding, and reproducibility	• Binds any biotinylated Ab • For samples high in soluble IgGs • Recombinant Ab lacking the Fc region

* Note that the SB buffer supplied in the kit contains Thermo Scientific™ Tween™ detergent, so the SB buffer will need to be replaced with standard TBS or PBS buffer.

Ordering information

Description	Quantity	Cat. No
Pierce™ MS-Compatible Streptavidin Magnetic IP Kits	40 reactions	90408
Pierce™ MS-Compatible Protein A/G Magnetic IP Kits	40 reactions	90409

Pierce Antibody Biotinylation Kit for IP

Optimized antibody biotinylation kits for IP and co-IP applications

The Thermo Scientific™ Pierce™ Antibody Biotinylation Kit for IP provides biotinylation reagents designed specifically for the labeling of primary antibodies used in IP applications.

The Pierce Antibody Biotinylation Kit reagents have been optimized and validated to biotinylate antibodies for IP and co-IP reactions. Determining the optimal number of biotins to attach to the target molecule is one of the major challenges of biotinylation. For IP and co-IP applications, too many biotins result in reduced affinity for the target antigen, while too few biotins result in antibody leaching upon elution of the target antigen. The biotin labeling procedure in the Pierce™ Antibody Biotinylation Kit for IP has been developed to address this challenge.

Highlights

- **Optimized**—reagents and protocols developed for efficient antibody biotinylation for IP applications
- **Easy to use**—kit contains all reagents and accessories to label and clean up 50–200 µg of antibody
- **Enhanced solubility**—pegylated linker improves the solubility of the biotinylated antibody and reduces aggregation
- **Improved binding**—longer spacer arm (29 angstroms) on biotinylation reagent minimizes steric hindrance when binding to avidin molecules

The kit contains sufficient reagents to label 50–200 µg of antibody in 100 µL reaction volumes for eight samples. The NHS-PEG_4-Biotin labeling reagent contains an amine-reactive N-hydroxysuccinimide ester (NHS) group and a water-soluble PEG_4 spacer for optimal labeling and is provided in easy-to-use, single-use microtubes. Both the labeling efficiency of the biotinylation reagent and binding affinity of the labeled antibody have been validated using mouse monoclonal (IgG1, IgG2), rabbit polyclonal, and rabbit monoclonal antibodies. Thermo Scientific™ Zeba™ Desalting Spin Columns are provided for easy and efficient removal of salts and excess biotin.

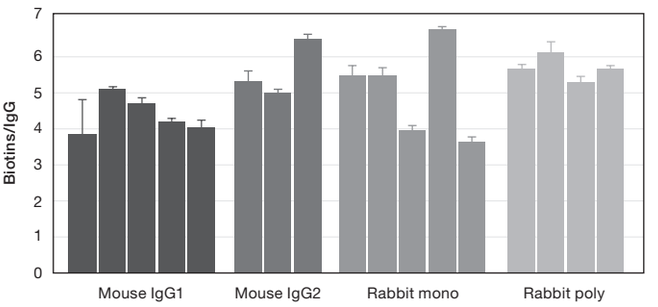


Figure 63. The Pierce Antibody Biotinylation Kit for IP enables effective labeling of multiple antibody types. The kit was used to label 17 different specific antibodies including mouse IgG1, mouse IgG2, rabbit monoclonal, and rabbit polyclonal. Biotinylation with this kit resulted in 3–7 biotins per IgG molecule as determined by the Thermo Scientific™ Pierce™ Fluorescent Biotin Quantitation Kit.

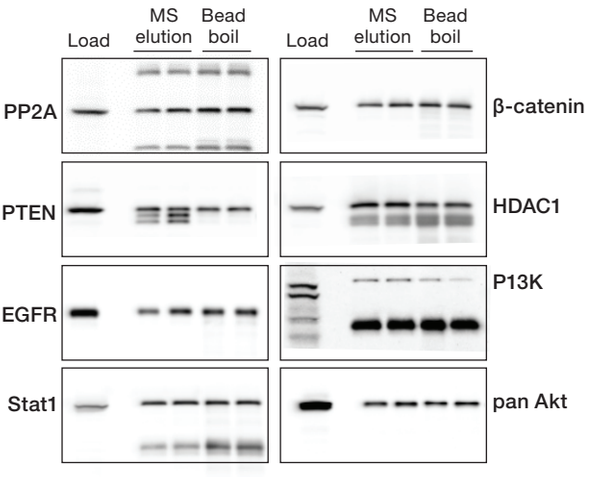


Figure 64. The Pierce Antibody Biotinylation Kit for IP allows effective target capture and elution. Antibodies were labeled with the kit and used in the Thermo Scientific™ Pierce™ MS-Compatible Magnetic IP Kit (Streptavidin) to IP target proteins from cell lysates. The elutions were analyzed by western blot.

Ordering information

Description	Quantity	Cat. No
Pierce™ Antibody Biotinylation Kit for IP	8 reactions	90407

Magnetic IP-MS kits and standards

Validated, modular reagents for multiplexed targeted protein quantitation

The Thermo Scientific™ SureQuant™ assays and standards have been designed for multiplexed, targeted quantitation of key cellular signaling proteins. The assays provide flexibility to use the immunoprecipitation (IP)sample prep kits and peptide quantitation modules together or independently based on the end application.

Features include:

- **Complete**—kits include reagents for successful sample preparation and quantitative analysis of each target peptide
- **Verified**—kits and reagents are rigorously tested for specificity and successful quantitation of each target peptide
- **Multiplex**—Thermo Scientific™ HeavyPeptide™ AQUA Custom Peptide Synthesis panels for simultaneous quantitation of target proteins and phosphorylation status from key cell signaling pathways
- **Flexible**—modular format allows for immunoenrichment only, or in combination with peptide quantitation panels

The Thermo Scientific™ SureQuant™ Protein A/G and Streptavidin IP-MS Sample Preparation Kits have been designed to support targeted quantitation using SureQuant or SRM/PRM analysis. The kits contain high-quality Thermo Scientific™ Pierce™ Protein A/G or Streptavidin Magnetic Beads together with reagents that have been optimized for MS compatibility. The streamlined procedure enables digestion immediately after the immunoprecipitation elution step, facilitating MS sample preparation in ~4 hours for

same-day LC-MS/MS analysis (Figure 65). These kits have also been rigorously validated using numerous target antigens with varying expression levels, including targets previously undetected by western blotting.

The Thermo Scientific™ SureQuant™ quantitation modules contain Thermo Scientific™ HeavyPeptide™ and/or LightPeptide AQUA Ultimate panels for multiplexed quantitation of target proteins using LC and MS. These peptide panels may be used for absolute quantitation by the generation of a standard curve or as a spike-in internal standard for relative quantitation.

Together, the SureQuant IP-MS sample preparation kits and the SureQuant quantitation modules provide a complete solution for multiplexed sample preparation and analysis of select target proteins (Figures 66 and 67).

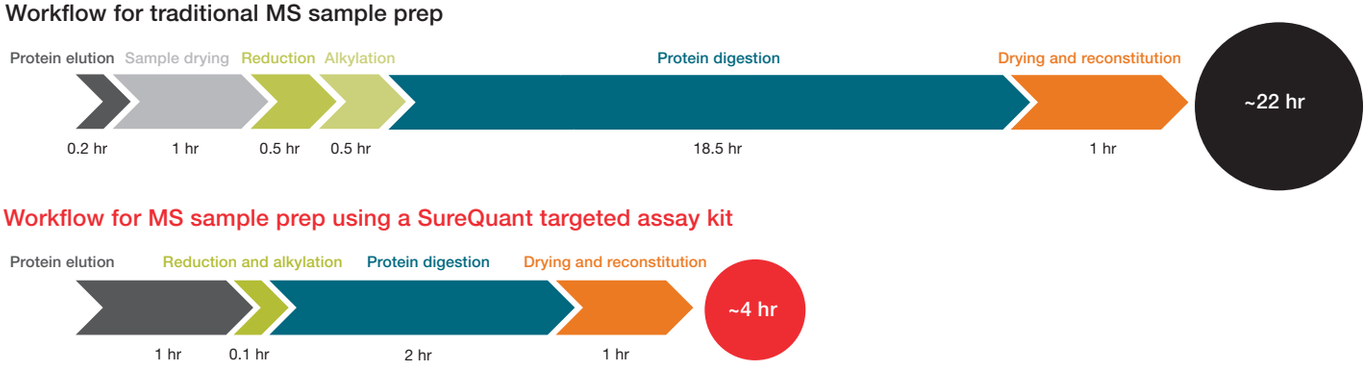


Figure 65. MS sample preparation procedure comparison between traditional methods and SureQuant targeted mass spec assay kits following immunoprecipitation

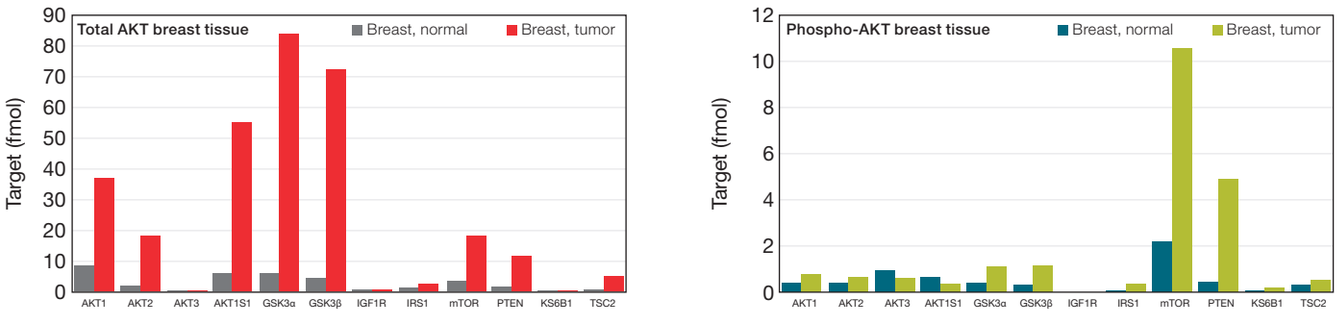


Figure 66. Multiplex IP analysis using streptavidin magnetic beads for human tissue. AKT/mTOR pathway proteins were enriched by multiplex IP using biotinylated antibodies from the Thermo Scientific™ SureQuant™ AKT Pathway kits. Parallel reaction monitoring (PRM) analysis for total AKT/mTOR pathway targets (left) and phosphorylated AKT/mTOR pathway targets (right) was performed using the Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLCnano System and Thermo Scientific™ Q Exactive™ HF Hybrid Quadrupole-Orbitrap™ Mass Spectrometer. Data were subsequently analyzed in Thermo Scientific™ Skyline software using calibration curves to determine the absolute protein amount (fmol).

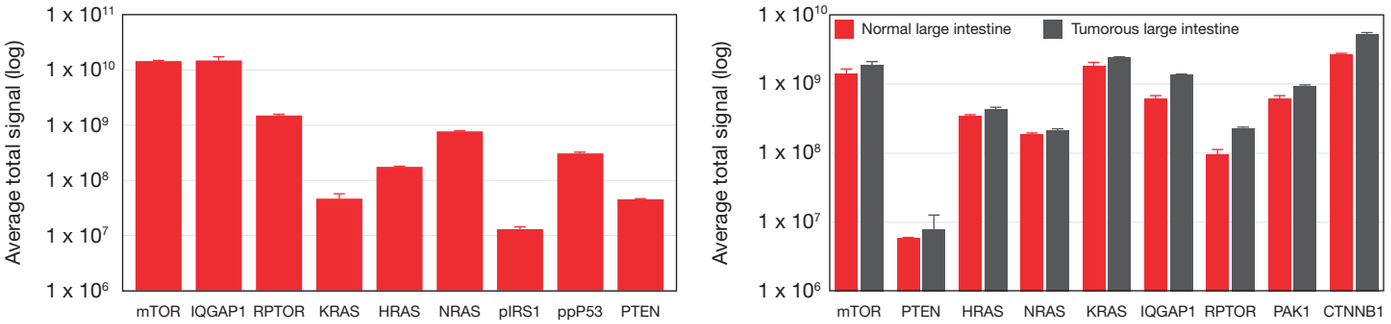


Figure 67. Multiplex IP analysis using Thermo Scientific™ Pierce™ Protein A/G Magnetic Beads for cell line and tissue lysates. Multiplex SureQuant™ Protein A/G IP-MS data using a lysate containing hIGF-1–treated MCF7 and HEK293 (left). Multiplex SureQuant™ Protein A/G IP-MS data for normal or tumorous large intestine human tissue (right). Data dependent acquisition (DDA) analysis was performed using the Dionex UltiMate 3000 RSLCnano System and Q Exactive HF Hybrid Quadrupole-Orbitrap Mass Spectrometer. Data were subsequently analyzed in Thermo Scientific™ Proteome Discoverer™ Software using the average total peptide area.

Ordering information

Description	Quantity	Cat. No
SureQuant Protein A/G IP-MS Sample Preparation Kit	20 reactions	A51743
SureQuant Streptavidin IP-MS Sample Preparation Kit	20 reactions	A51744

Find out more at thermofisher.com/ms-targeted-assays

KingFisher instruments

High-throughput quantitative proteomics

KingFisher instruments are versatile sample preparation instruments in the lab, and are elegantly designed to support multiple applications.

- **Various throughputs**—process 6–96 samples per run depending on the instrument model
- **Interchangeable formats**—choose 24- or 96-well plates so you can process a wide range of input volumes
- **Protocol customization**—easily edit, modify, or create new protocols (touchscreen enabled for Thermo Scientific™ KingFisher™ Apex instrument only)
- **Optimized reagents**—compatible with multiple magnetic-bead reagents
- **Barcoded plastics**—can help you achieve optimal performance with specially designed plastics (for KingFisher Apex instrument only)

Test a KingFisher platform in your lab

Specialists supporting the KingFisher instrument can provide an on-site demonstration of the system with your samples. They provide the instrument, consumables, and reagents from Thermo Fisher Scientific that are specific to your research needs. The demonstration includes instrument setup and use, protocol modification, and other FAQs.

Find out more at **thermofisher.com/kingfisherdemo**

Find a model that meets your needs




KingFisher instrument:	Duo Prime	Flex	Apex	Presto
Instrument size	Compact benchtop	Benchtop	Benchtop	Benchtop—integrates with robotic liquid handler
Throughput level	Low to medium	High	High	Ultrahigh
Processing volume range	• 50–1,000 µL: 12-pin magnet head • 200–5,000 µL: 6-pin magnet head	• PCR plate (20–100 µL*), skirted • 20–200 µL: 96-well plate • 50–1,000 µL: 96 deep-well plate • 200–5,000 µL: 24 deep-well plate	• 15–1,000 µL: 96 deep-well plate • 15–200 µL: 96-well KingFisher standard plate • 10–80 µL: 96-well PCR plate • 30–5,000 µL: 24 deep-well plate • 30–200 µL: 96 storage tubes • 200–1,000 µL: 24 storage tubes	• 50–1,000 µL: 96 deep-well plate • 200–5,000 µL: 24 deep-well plate • KingFisher 96 plate: 0–150 µL
Samples per run	6 or 12	24 or 96	24 or 96	24 or 96
Customizable protocols	Yes	Yes	Yes, with touchscreen or PC software	Yes
Heating/cooling	• 10°C to 75°C (plate row block A) • 4°C to 75°C (elution strip block)	From 5°C above ambient temperature to 115°C	• From 4°C above ambient temperature to 100°C • Cooling down to 4°C	From 5°C above ambient temperature to 115°C
Ultraviolet lamp	8 watts (up to 16 hr)	No	2 UV lamps, max 23 h 59 min	No
Additional details	For research use only	For laboratory use	For laboratory use	For laboratory use

* Or similar skirted PCR plate.

Find out more at **thermoscientific.com/kingfisher**

Learn more at **thermofisher.com/magbeadsproteomics**