CaptureSelect™ Biotin Conjugates

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

CaptureSelect™ biotin conjugates (or biotinylated affinity ligands) consist of a ~12-15 kDa recombinant single domain antibody fragment (V_hH affinity ligand) that specifically binds to the target of interest. The affinity ligand is chemically conjugated to biotin through an appropriate spacer. The spacer retains the binding reactivity of the ligand when it is immobilized on streptavidin-functionalized surfaces.

CaptureSelect™ biotinylated affinity ligands can be used for the specific detection, quantitation, and characterization of antibodies and antibody fragments, proteins, hormones and viral vectors. Targets can be obtained from complex sources such as plasma, serum, cell culture supernatants and cell lysates. Compatibility is demonstrated for a wide variety of detection platforms. The following demonstrated protocols are described in this guide:

- Sandwich ELISA
- Bio-layer interferometry
- Surface plasmon resonance
- Protein capture using magnetic beads
- Immunoprecipitation
- Western blot

All applications described in this guide are tested but not validated for the instrument system specified. Information is intended as a starting point for protocol development and optimization.

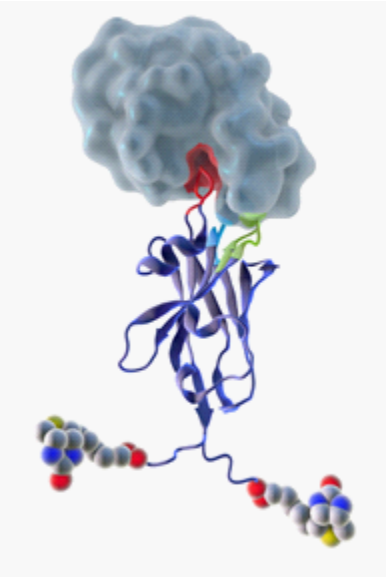

Fig. 1 Representation of a CaptureSelect™ biotin conjugate bound to its target protein. The biotin molecules are chemically conjugated to the affinity ligand through a spacer, allowing immobilization on streptavidin-functionalized surfaces.

Animal origin-free

CaptureSelect™ products contain affinity ligands based on recombinant single-domain antibody fragments (V_hH) created by a proprietary technology. The V_hH affinity ligand is a 12 to 15 kDa fragment comprising the three complementarity-determining regions (CDRs) that form the antigen-binding domain, efficiently produced in the yeast *Saccharomyces cerevisiae* by a production process free of any animal components.

Contents and storage

Contents	Amount	Storage
Biotin conjugate, 1 mg/ml protein in PBS, pH 7.4 (no preservatives added)	100 µg or 500 µg	• 4°C for short-term storage (up to 1 month) • –5°C to –30°C for long-term storage (aliquot to prevent repeated freeze/thaw cycles)

CaptureSelect™ biotinylated affinity ligands specifications

The following species abbreviations are used in this section:

Bv—Bovine
Ck—Chicken
Ct—Cat
CYN—Cynomolgus monkey
Cz—Chimpanzee
Dg—Dog
Do—Donkey
GP—Guinea pig
Gt—Goat
Hm—Syrian hamster
Hr—Horse
Hu—Human
Ms—Mouse
RM—Rhesus monkey
Rt—Rat
Rb—Rabbit
Sh—Sheep
Sw—Swine

Biotinylated affinity ligands for antibody detection

Table 1 CaptureSelect™ biotinylated affinity ligands for antibodies and antibody fragments

CaptureSelect™ affinity ligand	Target	Selectivity (binds to)	No cross binding with
Anti-IgG-CH1	CH1 domain of human IgG	• All Hu IgG subclasses (IgG1 – 4) including recombinant IgG • Total IgG in plasma or serum • Hu IgG Fab fragments • IgG of species: Ct, CYN, Cz, Dg, Do, GP, Hm, Hr, RM	• Hu IgA, IgD, IgE, and IgM • Free human light chains (kappa and lambda) • Ms and Bv IgG
Anti-IgG-Fc (human)	Fc part of human IgG (CH3 domain)	• Hu IgG 1-4 • Hu IgG Fc fragments • IgG of species: Rb, CYN, Cz, RM	• Hu IgA, IgD, IgE, and IgM • Free human light chains (kappa and lambda) • Fab fragments • IgG of species: Bv, Rt, Ms
Anti-IgG-Fc (rabbit)	Fc part of rabbit IgG	• Rb IgG from recombinant and plasma sources	• IgG of species: Hu, Ms, Rt, Bv, Hr, Sh, and Gt
Anti-IgG-Fc (multi-species)	Fc part of IgG from multiple species	• All IgG subclasses, including recombinant IgG and Fc-fusion proteins • Total IgG in plasma or serum • IgG of species: Hu, Ms, Rt, Rb, GP, Hm, Sw, Gt, Sh, Bv, Do, Dg, Ct, Cz, RM, CYN	• Hu IgA, IgD, IgE, and IgM • IgY
Anti-IgA	α- chain of human IgA (Fc domain)	• Hu IgA, recombinant or from plasma sources • Hu IgA Fc fragments • Monomeric IgA1 • Monomeric IgA2 • Dimeric IgA (secretory and non–secretory)	• Hu IgD, IgE, IgG, and IgM • Free light chains (kappa and lambda) • IgA of species: Ms, Rt, Bv
Anti-IgE	Fc-region (CH4 domain) of human IgE	• Hu IgE, recombinant or from plasma sources • Hu IgE Fc fragments	• Hu IgA, IgD, IgG, and IgM • Free light chains (kappa and lambda) • Rt IgE
Anti-IgM	μ-chain of IgM	• Hu, Ms, Rt IgM, recombinant or from plasma sources	• Hu IgA, IgD, IgE, and IgG • Free light chains (kappa and lambda) • Bv IgM
Anti-IgG3	Epitope within the IgG3 hinge region	• All human IgG3 subclass antibodies	• Hu IgA, IgD, IgE, and IgM • IgG1, 2, and 4 • Bv IgG
Anti-IgG4	Fc part of human IgG4	• All human IgG4 subclass antibodies	• Hu IgA, IgD, IgE, and IgM • IgG1, 2, and 3 • Bv IgG
Anti-LC-lambda (human)	Constant domain of human lambda light chains	• Hu IgG1- 4 • IgA, IgD, IgM, IgE • Fab fragments (recombinant and native) of all human antibody isotypes and subclasses containing a lambda light chain • Lambda light chains of antibody isotypes from species: Hu, Bv, Gt, Cz, RM, CYN	• Hu IgG Fc • Antibodies and fragments containing a kappa light chain • Free kappa light chains • IgG of species: Ms, Rb, Sh
Anti-LC-lambda (mouse)	Constant domain of mouse lambda light chains	• Ms IgG (all subclasses) • Fab fragments (recombinant and native) of all mouse antibody IgG subclasses containing a lambda light chain	• Hu, Rt IgG • Ms IgG-Fc • Antibodies and fragments containing a kappa light chain • Free kappa light chains
Anti-LC-kappa (human)	Constant domain of human kappa light chains	• Hu IgG1–4 • IgA, IgD, IgM, IgE • Fab fragments (recombinant and native) of all human antibody isotypes and subclasses containing a kappa light chain • Kappa light chains of antibody isotypes from species: Hu, Cz, RM, CYN	• Hu IgG Fc • Antibodies and fragments containing a lambda light chain • Free lambda light chains • IgG of species: Bv, Gt, Ms, Rb, Rt, Sh
Anti-LC-kappa (murine)	Constant domain of murine kappa light chains	• Murine IgG1 • Ms IgG2a/b • Rt IgG2a/b/c • Murine IgG3 • Murine IgA • Murine IgM Including all Fab fragments thereof containing a kappa light chain	• Rt, Ms IgG Fc • Antibodies and fragments containing a lambda light chain • Free lambda light chains • IgG of species: Bv, Gt, Rb, Sh

CaptureSelect™ affinity ligand	Target	Selectivity (binds to)	No cross binding with
Anti-Free LC Kappa (human)	Epitope on human CL-kappa domain that is masked in intact antibodies and Fab fragments	• Free human kappa light chains	• Human kappa light chains of intact antibodies and Fab fragments of any human isotype (IgA, IgD, IgE, IgG, IgM)

Biotinylated affinity ligands for studying antibody binding kinetics

CaptureSelect™ human kinetics biotin conjugates are bi-functional ligand constructs consisting of 2 different recombinant single domain antibody fragment (V_hH affinity ligand). These conjugates enable high-affinity capture of Human IgG Fab fragments that contain a kappa or lambda light chain. The bi-functional ligands are chemically conjugated to biotin, which allows immobilization on streptavidin-functionalized surfaces. They can be used for the screening of biomolecular interactions between Fab fragments and their specific target antigen. The kinetics ligands bind the CL-kappa or CL-lambda (light chain) and the CH1 domain (heavy chain) of a human IgG Fab fragment simultaneously. This binding results in extreme low dissociation rates (Kd) between the ligand and the captured Fab fragment.

The CaptureSelect™ Human IgG-Fc PK Biotin Conjugate consists of a 13 kDa recombinant single domain antibody fragment (V_hH affinity ligand), chemically conjugated to biotin. The ligand specifically binds to the Fc part of all four human IgG subclasses. The conjugate can be used for the detection, quantification and characterization of all human (recombinant) IgG antibodies and Fc-fusion proteins in, for example, non-human plasma and/or serum samples like mouse, rat, rhesus, and cynomolgus monkey. This makes the conjugate extremely suitable for setting up pharmacokinetics (PK) assays.

Table 2 CaptureSelect™ biotinylated affinity ligands to study human kinetics

CaptureSelect™ affinity ligand	Target	Selectivity (binds to)	No cross binding with
Human Fab-kappa kinetics (bi-functional affinity ligand)	• Constant domain of human kappa light chains • CH1 domain of human IgG	• Simultaneous detection of CL-kappa domain and CH1 domain of Human IgG Fab fragments	• Hu IgG-Fc • IgG from Ms or Bv sources • Antibodies and fragments containing a lambda light chain • Free lambda light chains
Human Fab-lambda kinetics (bi-functional affinity ligand)	• Constant domain of human lambda light chains. • CH1 domain of human IgG	• Simultaneous detection of CL-lambda domain and CH1 domain of Human IgG Fab fragments	• Hu IgG-Fc • IgG from Ms or Bv sources • Antibodies and fragments containing a Kappa light chain • Free kappa light chains
Human IgG-Fc PK	• CH2 domain of human IgG	• Fc part of all 4 human IgG subclasses (IgG1–4)	• Non-human IgG antibodies, except Cz IgG

Biotinylated affinity ligands for proteins and hormone detection

Table 3 CaptureSelect™ biotinylated affinity ligands for proteins and hormones

CaptureSelect™ affinity ligand	Target	Binding characteristics
Anti-AAT	Human α-antitrypsin	Binds native and recombinant human α-antitrypsin (AAT)
Anti-C1-Inhibitor	Human C1 esterase inhibitor	Binds native and recombinant human C1 esterase inhibitor (C1-Inh)
Anti-EPO	Human erythropoietin	Binds native and recombinant human erythropoietin (EPO)
Anti-C-tag[1]	4 amino acid C-tag peptide tag: E-P-E-A	Binds the E-P-E-A sequence when this tag is directly fused to the C-terminus of a protein.
Anti-FVII	Human Factor VII	Binds recombinant and plasma derived human factor VII including the activated form (FVIIa)
Anti-FVIII	Human Factor VIII	Binds recombinant (full length and B-domain deleted) and plasma derived Human Factor VIII (FVIII)
Anti-FIX	Human Factor IX	Binds recombinant and plasma derived human factor IX (FIX)
Anti-Fibrinogen	Human fibrinogen	Native and recombinant human fibrinogen (coagulation factor I, FI)
Anti-FSH	Human follicle stimulating hormone	Binds native and recombinant human follicle stimulating hormone (FSH), through recognition of an epitope specific for intact FSH only
Anti-Gonadotropin	Alpha chain of human gonadotropins	Binds native and recombinant human gonadotropins through recognition of the alpha chain subunit. • Human chorionic gonadotropin (HCG) • Luteinizing hormone (LH) • Thyroid-stimulating hormone (TSH) • Follicle stimulating hormone (FSH)
Anti-hGH	Human growth hormone	Binds native and recombinant human growth hormone (hGH)
Anti-Human Albumin (HSA)	Human serum albumin	Binds native and recombinant human serum albumin (HSA), including albumin fusion proteins
Anti-Prothrombin	Human prothrombin	Binds native and recombinant human prothrombin (coagulation factor II, F2)
Anti-Transferrin	Human transferrin	Binds native and recombinant human transferrin

[1] C-tag is a small, four-amino-acid peptide tag: EPEA (glutamic acid–proline–glutamic acid–alanine), which enables simple detection of C-tagged fusion proteins.

Biotinylated affinity ligands for detection of viral vectors

Table 4 CaptureSelect™ biotinylated affinity ligands for viral vectors

CaptureSelect™ affinity ligand	Target	Binding characteristics
Anti-AAV8	Adeno-associated virus particles, serotype 8	Preferential binding to AAV8 virus particles
Anti-AAV9	Adeno-associated virus particles, serotype 9	Preferential binding to AAV9 virus particles
Anti-AAVX	Adeno-associated virus particles, various serotypes	Binds to a wide selection of AAV virus particles: AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVrh10
Anti-Adv5	Adenovirus serotype 5	Binds to recombinant hexon capsomeres of Adenovirus serotype 5 (AdV5) particles as well as free hexon proteins. Cross-binding to other Adenovirus serotypes not tested.

Sandwich ELISA procedure and application examples

Note: Optimize the procedures in this section for your application.

Sandwich ELISA procedure

- **Buffer**—PBS, 1% (w/v) BSA, 0.05% (v/v) Tween® 20
- **Plates**—Nunc™ MaxiSorp™ flat-bottom 96-well plates
- **Detection antibody**—Monoclonal or polyclonal antibody against the target of interest that is conjugated to an enzyme such as horse radish peroxidase (HRP).

1. Coat 96-well plates with 1 µg/mL of streptavidin in PBS, 100 µL/well, and incubate for 1 hour at room temperature or overnight at 4°C.
2. Prepare CaptureSelect™ biotin conjugate (5 µg/mL in buffer), then add 100 µL/well to streptavidin-coated plates. Incubate for 1 hour at room temperature to immobilize.
3. Prepare a dilution series of the target of interest. Add 100 µL/well of each concentration to the plates. Incubate for 1 hour at room temperature.
4. To detect bound target of interest, use commercially available detection antibodies (for example, antibodies conjugated to HRP.
5. Generate a coloured readout with TMB/H_20_2-based substrates or equivalent substrates suitable for HRP.

Sandwich ELISA application example—IgG3

When immobilized on streptavidin-coated microtiter plates, CaptureSelect™ biotin anti-IgG3 (Hu) can be used as a capturing agent in sensitive assays to specifically detect and quantitate human IgG 3, without cross-binding with other subclasses. See Figure 2.

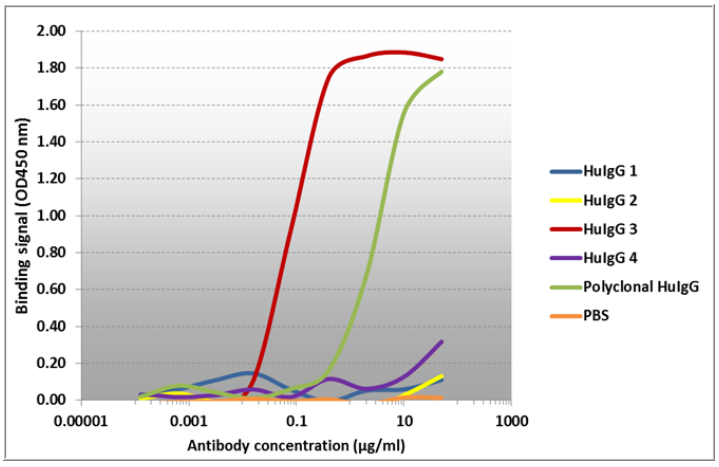

Fig. 2 Example dose-response curves for human IgG 1, 2, 3, 4 and polyclonal human IgG in Capture ELISA using Biotin Anti-IgG3 (Hu) as the capturing agent. The subclass specific samples have a purity of approximately 98–99%.

Label-free and real-time binding assays

The CaptureSelect™ biotinylated affinity ligands can be used in label-free and real-time binding assays such as Bio-Layer Interferometry (BLI) and Surface Plasmon Resonance (SPR).

Both systems provide streptavidin-linked biosensors that can immobilize the biotinylated ligands. The biotinylated affinity ligands can be used as the capturing agent to measure interactions of proteins and/or antibodies and antibody subtypes. Samples can be from complex sources such as plasma, serum, and cell culture supernatants.

Bio-Layer Interferometry (BLI) procedure and application example

Note: Optimize the procedures in this section for your application.

BLI procedure

- **Hydration buffer**—PBS
- **Running buffer**—PBS, 0.1% (w/v) BSA, 0.005% (v/v) Tween® 20
- **Regeneration solution**—0.1 M Glycine, pH 2

1. Load prepared CaptureSelect™ biotin conjugate (5 µg/mL in 200 µL running buffer) on ForteBio™ streptavidin-linked biosensors (SA or SAX) for 5 minutes at a shake speed of 1,000 rpm, then wash with running buffer for 1 minute.
2. Bind the target protein of interest (in running buffer) for 10 minutes at a shake speed of 1,000 rpm, then dissociate with running buffer for 10 minutes.
3. Regenerate the biosensors with regeneration solution. Use 3 regeneration cycles (5 sec regeneration/ 5 sec neutralization) at a shake speed of 1,000 rpm.

BLI application example—FSH

CaptureSelect™ biotin anti-FSH is highly compatible with ForteBio™ Streptavidin (SA) Biosensors, and can be used in a range of applications for antibody analytics on the Octet™ platform. See Figure 3 and Figure 4.

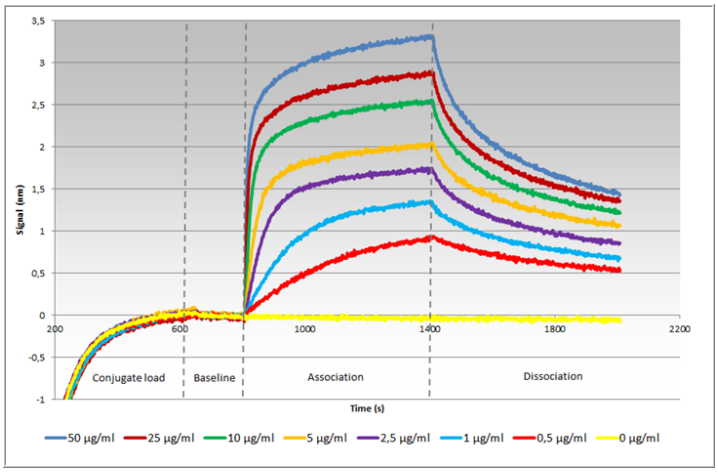

Fig. 3 Binding analysis of recombinant human FSH on Streptavidin (SA) Biosensors (Octet™ system) functionalized with biotin anti-FSH showing association and dissociation of recombinant human FSH at different concentrations.

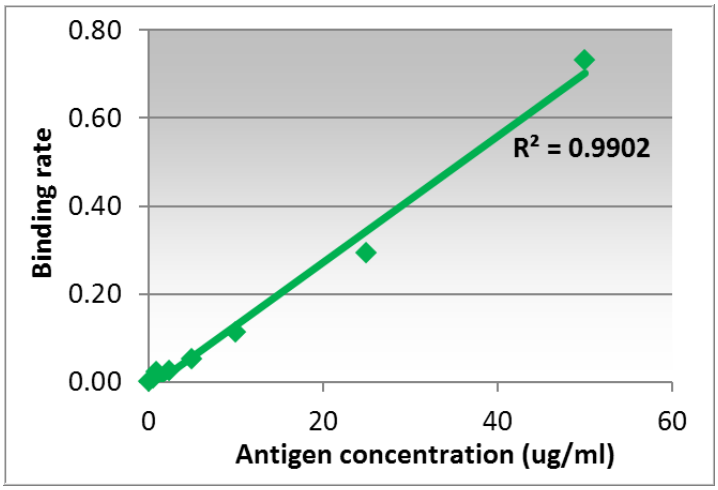

Fig. 4 Example calibration curve of recombinant FSH on biotin-anti-FSH-functionalized biosensors. To demonstrate the use of CaptureSelect™ biotin anti-FSH for quantitation purposes, binding rates for the first 10 seconds of association were obtained.

Surface Plasmon Resonance (SPR) procedure and application examples

Note: Optimize the procedures in this section for your application.

SPR procedure: SA sensor chip

For more information on Biacore™ assay setup, go to **http://www.cytivalifesciences.com**.

- **Running Buffer**—HBS-EP buffer
- **Regeneration solution**— 0.1 M Glycine, pH 2

1. Load prepared CaptureSelect™ biotin conjugate (5 µg/mL in running buffer) onto a SA sensor chip (Cytiva BR100398) at a flow rate of 10 µL/minute for at least 3 minutes.
2. Bind the protein target of interest (for example, 10 µg/mL in HBS-EP buffer) at a flow rate of 5 µL/minute for 1 minute.
3. Dissociate in running buffer at a flow rate of 5 µL/minute for 2.5 minutes.
4. Regenerate after each cycle with regeneration solution at a flow rate of 30 µL/minute for 1.5 minutes.

SPR application example—SA sensor chip IgG-CH1 application example

CaptureSelect™ biotin anti-IgG-CH1 is compatible with the Sensor Chip SA (Figure 5) and the Biotin CAPture Kit, which enables reversible capture of biotinylated molecules and standardized regeneration for interaction studies.

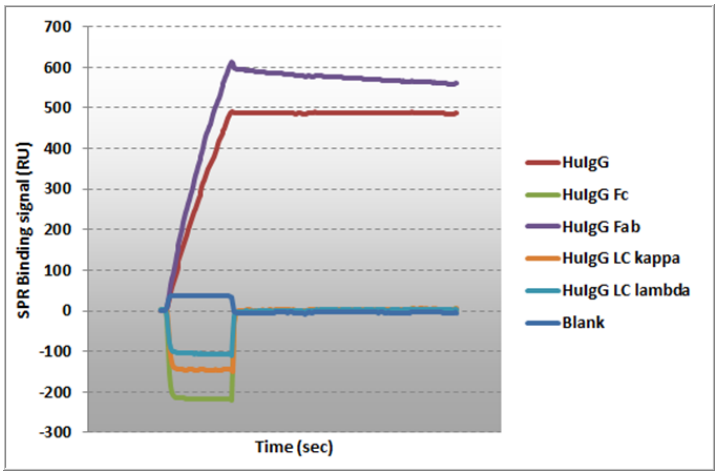

Fig. 5 Association and dissociation curves of polyclonal human IgG antibodies and Fab/Fc fragments on Sensor Chips SA (Biacore™ 3000 system) functionalized with biotin anti-IgG-CH1. The IgG-CH1 affinity ligand specifically binds to the CH1 domain of all human IgG subclasses. Therefore, positive binding to human IgG (HuIgG) and a human Fab fragment (HuIgG Fab) are observed. The antibody fragments without CH1 domain show a negative binding signal.

SPR procedure: IBIS MX96

For more information on Instrument for Biomolecular Interaction Sensing MultipleX 96 (IBIS-MX96) assay setup, go to **www.ibis-spr.nl**.

- **Running buffer**—PBS, 0.075% (v/v) Tween® 20
- **Regeneration solution**—0.1 M Glycine, pH 2

1. Prepare a dilution series of CaptureSelect™ biotin conjugate in running buffer.
2. Using a continuous flow microspotter (**https://www.ibis-spr.nl/product/cfm-printer/**), spot each concentration onto a SensEye™ sensor at a flow rate of 45 µL/minute for 20 minutes.
3. Bind the protein target of interest (for example, 10 µg/mL in running buffer at a continuous forward and reverse flow for 3 minutes.
4. Dissociate in running buffer at a flow rate of 8 µL/minute for 2.5 minutes.
5. Regenerate after each cycle with 0.1 M glycine, pH 2, for 1 minute.

SPR application example—IBIS MX96–hGH

CaptureSelect™ biotin anti-HGH is highly compatible with SensEye™ SPR sensors, and can be used in a range of applications for antibody analytics on the IBIS MX96 platform. It is possible to generate 9,216 interactions in an overnight run. Spotting multiple CaptureSelect™ biotinylated affinity ligands creates a diverse surface for screening on selectivity. See Figure 6.

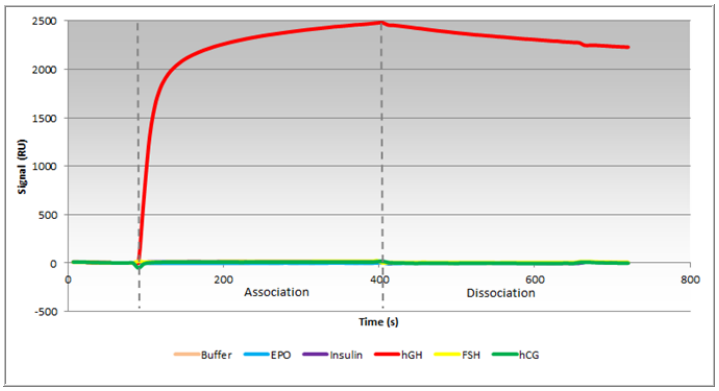

Fig. 6 Binding analysis of recombinant protein samples on a SensEye™ sensor functionalized with biotin anti-hGH showing association and dissociation steps. CaptureSelect™ biotin anti-hGH specifically binds human growth hormone and shows no cross-binding with other proteins.

Protein capture with magnetic beads guidelines and procedure

Note: Optimize the procedures in this section for your application.

Protein capture with magnetic beads guidelines

The CaptureSelect™ biotin conjugates can be used in combination with streptavidin-coupled magnetic beads. For simple and effective immobilization, use any of the following products available from Thermo Fisher Scientific:

- Dynabeads™ M-280 Streptavidin
- Pierce™ Streptavidin Magnetic Beads
- Dynabeads™ MyOne™ Streptavidin C1Dynabeads™ and Dynabeads™ MyOne™ Streptavidin T1

Magnetic beads coated with CaptureSelect™ biotin conjugates can be used as the capturing agent to measure interactions of proteins, antibodies, and antibody subtypes. Samples can be from complex sources such as plasma, serum, and cell culture supernatants.

Binding capacity can be affected by the size of the molecule to be targeted and by the level of biotin-conjugate labeling of the streptavidin beads.

Protein capture with magnetic beads procedure

- **Wash buffer**—PBS, 1% (w/v) BSA, 0.05% (v/v) Tween® 20.
- **Elution buffer**—0.1 M Glycine, pH 2
- **Neutralization buffer**—1 M Tris pH 9.0

1. Wash and pre-coat the Streptavidin-coupled Magnetic Beads.
   a. Resuspend the Magnetic Beads in the vial (vortex for >30 seconds or tilt and rotate for 5 minutes).
   b. Transfer the desired volume of Magnetic Beads to a tube.
   c. Add an equal volume of wash buffer (minimum of 1 mL), then mix (vortex for 5 seconds, or place on a roller apparatus for at least 5 minutes).
   d. Place the tube on a magnet for 2–3 minutes, then discard the supernatant.
   e. Remove the tube from the magnet, then resuspend the washed Magnetic Beads in the same volume of buffer as the initial volume of Magnetic Beads taken from the vial (substep 1b.)
   f. Add the CaptureSelect™ biotin conjugate to the washed magnetic beads. Use the bead manufacturers' suggested concentration of conjugate.
   g. Incubate for 15–30 minutes at room temperature with gentle rotation of the tube.
   h. Place the tube on a magnet for 2–3 minutes, then discard the supernatant.
   i. Wash the coated beads 3–4 times in wash buffer.
   j. Resuspend the coated beads to the desired concentration. Use a suitable buffer for downstream use. Store the resuspended beads at 4°C or discard the supernatant and continue to the capture step.
2. Capture the protein/antibody of interest.
   a. Add the sample solution containing the protein/antibody of interest to the desired volume of magnetic beads from substep 1j.
   b. Incubate for 15–30 minutes at room temperature with gentle rotation of the tube.
   c. Wash the sample 3–4 times in Wash buffer.
   d. Place the tube on a magnet for 2–3 minutes, then discard the supernatant.
   e. Add an appropriate volume of elution buffer.
   f. Incubate for 15–30 minutes at room temperature with gentle rotation of the tube.
   g. Place the tube in a magnet for 2–3 minutes, then collect the eluate.
   h. Adjust the pH of the eluate by adding 20% neutralization buffer (for example, 100 µL eluate + 20 µL neutralization buffer).
3. To wash and store the magnetic beads:
   a. Wash the magnetic beads using a recommended wash protocol.
   b. Place the tube on a magnet for 2–3 minutes, then discard the supernatant.
   c. Resuspend the magnetic beads in an appropriate volume of 20% ethanol.
   d. Store at 4°C.

Immunoprecipitation (IP) guidelines and procedure

Note: Optimize the procedures in this section for your application.

IP guidelines

Immunoprecipitation (IP) is one of the most widely used immunochemical techniques. Immunoprecipitation followed by SDS-PAGE and immunoblotting is routinely used in a variety of applications:

- Determine the molecular weights of protein antigens
- Study protein/protein interactions
- Determine specific enzymatic activity
- Monitor protein post-translational modifications
- Determine the presence and quantity of proteins

In the IP method, the protein from the cell or tissue homogenate is precipitated in an appropriate lysis buffer. Precipitation is achieved using an immune complex that contains the antigen (protein), CaptureSelect™ biotinylated affinity ligand, and streptavidin agarose.

Immunoprecipitation procedure

- Buffer—PBS
- Pierce™ Streptavidin Agarose (Cat. No. 20347) **thermofisher.com/order/catalog/product/20347?SID=srch-srp-20347**
- Novex™ Tris-Glycine SDS Sample Buffer (2X) (Cat. No. LC2676) **thermofisher.com/order/catalog/product/LC2676?SID=srch-srp-LC2676**

1. Wash and pre-coat the Streptavidin Agarose.
   a. Wash the streptavidin agarose beads two times with buffer, then centrifuge for 10 seconds at 12,000 × *g*. Discard the supernatant.
   b. Resuspend streptavidin agarose beads in buffer (50% suspension).
   c. Divide the streptavidin agarose beads into aliquots of 50–100 µL (~25–50 µL agarose/bed volume) in microcentrifuge tubes.

d. To each tube, add 10 µL of CaptureSelect™ biotin conjugate at an optimized dilution.
e. Incubate for 60 minutes at room temperature while gently mixing the suspension on a suitable shaker.
f. Centrifuge at 3,000 × *g* for 2 minutes at 4°C. Discard the supernatant.
g. To each tube, add 1 mL buffer, then centrifuge at 3,000 × *g* for 2 minutes at 4°C.
h. Repeat substep 1g at least two times.

2. Capture the protein/antibody of interest.
   a. To each tube, add 0.1–1.0 mL of cell lysate.
   b. Incubate for 90 minutes to overnight at 4°C, while gently mixing the sample on a suitable shaker.
   c. Collect the immunoprecipitated complexes by centrifugation at 3,000 × *g* for 2 minutes at 4°C. Discard the supernatant.
   d. Wash the pellet with 1 mL buffer, centrifuge at 3,000 × *g* for 2 minutes at 4°C.
   e. Repeat substep 2d at least 3 times.
3. Prepare the pellets and run SDS-PAGE.
   a. Resuspend each pellet in 25–100 µL Tris-Glycine SDS Sample Buffer (2X) to a final concentration of 1X sample buffer. Heat the samples at 95°C for 5 minutes.
   b. Run the samples and molecular weight standards with known concentrations on SDS-PAGE. Use an appropriate percentage of polyacrylamide gel for the molecular size of the protein.
   c. Transfer the samples to a nitrocellulose membrane, then perform immunoblotting.

Western blot: Guidelines, procedure, and application example

Note: Optimize the procedures in this section for your application.

Western blot guidelines

CaptureSelect™ biotin conjugates that are directed against antibodies can be used in Western blot applications under non-reducing conditions. Reducing conditions can be used for:

- CaptureSelect™ biotin anti-free LC-kappa (Hu)
- CaptureSelect™ biotin anti-C-tag
- CaptureSelect™ biotin anti-IgG-Fc (Hu)

The Western blot application has not been tested for biotin conjugates directed against non-antibody protein and viral vector targets.

Western blot procedure

- **Buffer**—PBS, 1% (w/v) skimmed milk, 0.05% (v/v) Tween® 20
- **Detection antibody**—Streptavidin Protein, AP (Cat. No. 21323) **thermofisher.com/antibody/product/Streptavidin-Protein/21323**
- **Detection substrate**—NBT/BCIP substrate

1. Run the protein sample(s) of interest by SDS PAGE, then transfer the separated proteins onto an appropriate membrane using an appropriate transfer method (for example, electroblotting).
2. Block the membrane for 1 hour at room temperature with 2% (w/v) skimmed milk in PBS.
3. Incubate the blocked membrane with CaptureSelect™ biotin conjugate (1 µg/mL) in buffer.
4. Detect bound CaptureSelect™ biotin conjugate using Streptavidin Protein AP (2 µg/mL).
5. Generate a color reaction with NBT/BCIP-based substrates or equivalent substrates suitable for alkaline phosphatase.

Western blot application example—C-tag

In combination with commercially available Streptavidin-AP conjugates, Biotin Anti-C-tag can be used in Western blot for the detection and quantitation of C-tag fusion proteins. See Figure 7.

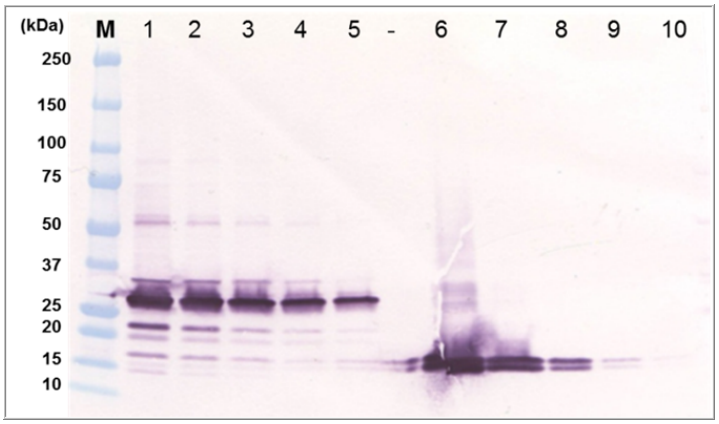

Fig. 7 Western blot analysis of GFP (crude lysate) and a single domain antibody (purified) both equipped with the C-tag peptide.

- M: Pre-stained marker
- 1: GFP-EPEA fusion, crude E.coli lysate, 20X dilution
- 2: 40X dilution
- 3: 80X dilution
- 4: 160X dilution
- 5: 320X dilution
- 6: Single-domain antibody-EPEA fusion, 400 ng/mL
- 7: 80 ng/mL
- 8: 15 ng/mL
- 9: 3.0 ng/mL
- 10: 0.6 ng/mL

Ordering information

CaptureSelect™ Biotin Conjugate	Cat. No. 100 µg	Cat. No. 500 µg
CaptureSelect™ biotin-conjugated ligands for antibodies and antibody fragments		
Anti-IgG-CH1	7103202100	7103202500
Anti-IgG-Fc (human)	7103262100	7103262500
Anti-IgG-Fc (rabbit)	7103642100	7103642500
Anti-IgG-Fc (multi-species)	7102852100	7102852500
Anti-IgA	7102882100	7102882500
Anti-IgE	7103542100	7103542500
Anti-IgM	7102892100	7102892500
Anti-IgG3 (human)	7103042100	7103042500
Anti-IgG4 (human)	7102902100	7102902500
Anti-LC-lambda (human)	7103082100	7103082500
Anti-LC-lambda (mouse)	7103232100	7103232500
Anti-LC-kappa (human)	7103272100	7103272500
Anti-LC-kappa (murine)	7103152100	7103152500
Anti-Free LC-kappa (human)	7103292100	7103292500
CaptureSelect™ biotin-conjugated ligands for kinetic experiments		
Human Fab-kappa Kinetics	7103302100	7103302500
Human Fab-lambda Kinetics	7103312100	7103312500
Human IgG-Fc PK	7103322100	7103322500

CaptureSelect™ Biotin Conjugate	Cat. No. 100 µg	Cat. No. 500 µg
CaptureSelect™ biotin-conjugated ligands for therapeutic proteins and hormones		
Anti-AAT	7102872100	7102872500
Anti-C1-Inhibitor	7103402100	7103402500
Anti-EPO	7103372100	7103372500
Anti-C-tag[1]	7103252100	7103252500
Anti-FVII	7102992100	7102992500
Anti-FVIII	7102862100	7102862500
Anti-FIX	7103002100	7103002500
Anti-FX	7103702100	7103702500
Anti-Fibrinogen	7102912100	7102912500
Anti-FSH	7103180100	7103180500
Anti-Gonadotropin	7103412100	7103412500
Anti-HGH	7103160100	7103160500
Anti-HSA	7102972100	7102972500
Anti-Prothrombin	7103452100	7103452500
Anti-Transferrin	7103062100	7103062500
CaptureSelect™ biotin-conjugated ligands for viral vectors		
Anti-AAV8	7103382100	7103382500
Anti-AAV9[2]	7103332100	7103332500
Anti-AAVX[2]	7103522100	7103522500
Anti-AdV5	7103532100	7103532500

[1] Product also available as Alexa Fluor-labeled.

[2] Products also available as HRP-labeled.

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Revision	Date	Description
B.0	4 May 2022	• Update to the V_hH affinity ligand. • Addition of “Animal origin-free” on page 1.
A.0	13 April 2021	New document that describes the use and application of all CaptureSelect™ biotin conjugate products. This document replaces the following product information sheet publication numbers: MAN0014341, MAN0014342, MAN0014343, MAN0010059, MAN0010060, MAN0010061, MAN0010062, MAN0010063, MAN0010064, MAN0010065, MAN0010066, MAN0010067, MAN0010707and 4485775.