invitrogen

Dynabeads™ Protein G

Catalog Nos. 10003D, 10004D, 10009D **Store at 2°C to 8°C**

Publication No. MAN0015809 **Rev. Date:** 18 March 2016 (Rev. A.0)

Product contents

Cat. No.	Volume
10003D	1 mL
10004D	5 mL
10009D	50 mL

Dynabeads™ Protein G contains 30 mg/mL of beads in phosphate buffered saline (PBS), pH 7.4, with 0.01% Tween™ 20 and 0.09% sodium azide as a preservative.

Product description

Dynabeads™ Protein G is designed for immunoprecipitation of proteins, protein complexes, protein-nucleic acid complexes, and other antigens.

Antibodies (Ab) ard added to the Dynabeads™ Protein G, and bind to the magnetic beads via their Fc-region during a short incubation. The tube is placed on a magnet, and the beads adhere to the side of the tube facing the magnet, allowing easy removal of the supernatant.

The bead-bound Ab is then used for immunoprecipitation. Bound material is easily collected utilizing the unique magnetic properties of the Dynabeads™ magnetic beads.

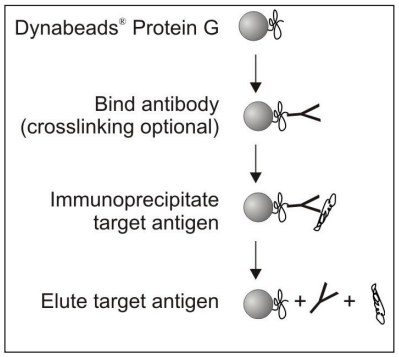

Figure 1 Principle of immunoprecipitation of antigen using Dynabeads™ Protein G.

Required materials

- DynaMag™ Magnet
  (See **thermofisher.com/magnets** for recommendations)
- Sample mixer allowing tilting and rotation of tubes (e.g. HulaMixer™ Sample Mixer)

Buffers and solutions

The following reagents are general recommendations. Alternative buffers may also be used. See "General guidelines" for additional details.

- Cell lysis buffer (e.g. Cell Extraction Buffer or NP-40 Cell Lysis Buffer)
- PBS pH 7.4 with and without 0.02% Tween™ 20
- 50 mM glycine pH 2.8 (elution buffer)
- NuPAGE™ LDS Sample Buffer and NuPAGE™ Sample Reducing Agent (elution buffer).

General guidelines

- Dynabeads™ Protein G have a binding capacity of ~8 µg of human IgG per mg of beads. The amount of Ab captured depends on the concentration of beads and Ab in the starting sample, as well as the type of immunoglobulin being bound (see Table 1).
- For standard immunoprecipitation use PBS for antibody binding and washing steps. Other possible buffers include alternative phosphate buffers, HEPES, Tris, and lysis buffer (e.g. RIPA, NP40). Elution buffer may also be substituted by alternative low pH, high pH, or high salt buffers, depending on the application.
- An incubation time of 10 minutes for immunoprecipitation is sufficient for most applications. Increasing the incubation time to 20–120 minutes can increase yield, particularly for low affinity antibodies, but may increase non-specific binding.
- For low-affinity antibodies, pre-incubate the sample and antibody prior to bead capture to improve binding kinetics for the antibody and minimize non-specific binding. This approach is also recommended when working with protein/nucleic acid complexes, e.g. ChIP.
- For sensitive proteins and phosphorylation studies, perform the isolation protocol and elution at 4°C to avoid protein complex dissociation and minimize enzymatic activity.

Protocol

This protocol provides a general procedure for immunoprecipitation. Optimization may be required for each antibody and target antigen. The protocol uses 50 µL of Dynabeads™ Protein G, but may be scaled up or down as required.

Lyse cells

Cells may be lysed using any standard cell lysis protocol compatible with your starting material. We recommend the use of Cell Extraction Buffer or NP40 Cell Lysis Buffer. For protocols and additional information about cell lysis, see **thermofisher.com/immunoprecipitation**.

Prepare Dynabeads™ magnetic beads

1. Resuspend Dynabeads™ magnetic beads in the vial (vortex >30 seconds or tilt and rotate 5 minutes).
2. Transfer 50 µL (1.5 mg) of Dynabeads™ magnetic beads to a tube.
3. Place the tube on the magnet to separate the beads from the solution, and remove the supernatant.
4. Remove the tube from the magnet.
5. Proceed directly to "Bind antibody".

Bind antibody

1. Add your antibody (typically 1–10 µg) diluted in 200 µL PBS with Tween™ 20, to the Dynabeads™ magnetic beads from step 4 in "Prepare Dynabeads™ magnetic beads". The optimal amount of Ab depends upon the individual Ab used.
2. Incubate with rotation for 10 minutes at room temperature.
3. Place the tube on the magnet and remove the supernatant.
4. Remove the tube from the magnet and resuspend the magnetic bead-Ab complex in 200 µL PBS with Tween™ 20. Wash by gentle pipetting.
   Note: Ab-conjugated Dynabeads™ magnetic beads can be stored in PBS (pH 7.4) with 0.01–0.1% Tween™ 20 to prevent aggregation.
5. Proceed to "Immunoprecipitate target antigen".

Crosslink antibody

To avoid co-elution of your antibody, crosslink your antibody to the Dynabeads™ magnetic beads before immunoprecipitation. Use the crosslinking reagent BS_3. For further information and procedure, visit **thermofisher.com/crosslinking**.

Immunoprecipitate target antigen

1. Place the tube (from step 4 of "Bind antibody") on the magnet and remove the supernatant.
2. Add your sample containing the antigen (Ag) (typically 100–1000 µL) and gently pipette to resuspend the magnetic bead-Ab complex.
3. Incubate with rotation for 10 min at room temperature to allow Ag to bind to the magnetic bead-Ab complex.
   Note: Depending on the affinity of the antibody, it may be necessary to increase incubation times for optimal binding.
4. Place the tube on the magnet. Transfer the supernatant to a clean tube for further analysis, if desired.

5. Wash the magnetic bead-Ab-Ag complex 3 times by gentle pipetting using 200 µL of Washing Buffer for each wash. Place the tube on the magnet and remove the supernatant between each wash.
6. Resuspend the magnetic bead-Ab-Ag complex in 100 µL of Washing Buffer and transfer the bead suspension to a clean tube to avoid co-elution of proteins bound to the tube wall.
   Note: To store the immunoprecipitated protein, add elution buffer and sample buffer, then freeze the magnetic bead-Ab-Ag complex. For subsequent analysis of the sample, thaw and continue with the elution protocol.
7. Proceed to "Elute target antigen".

Elute target antigen

Denaturing elution

1. Place the tube (from step 6 of "Immunoprecipitate target antigen") on the magnet and remove the supernatant.
2. Add 20 µL of Elution Buffer, and 10 µL of premixed NuPAGE™ LDS Sample Buffer and NuPAGE™ Sample Reducing Agent.
3. Gently pipette to resuspend the magnetic bead-Ab-Ag complex.
4. Heat for 10 minutes at 70ºC.
5. Place the tube on the magnet and load the supernatant/sample onto a gel.

Note: As an alternative, the magnetic bead-Ab-Ag complex can be resuspended in a different sample buffer of your choice (e.g. SDS sample buffer). Follow the recommended temperatures and heating times for these buffers prior to gel loading.

Non-denaturing elution

1. Place the tube (from step 6 of "Immunoprecipitate target antigen") on the magnet and remove the supernatant.
2. Add 20 µL Elution Buffer and gently pipette to resuspend the magnetic bead-Ab-Ag complex. Avoid foaming.
3. Incubate with rotation for 2 minutes at room temperature to dissociate the complex.
4. Place the tube on the magnet and transfer the supernatant containing eluted Ab and Ag to a clean tube. If the eluted protein is to be used for functional assays or stored, the pH of the eluate can be adjusted by adding 1 M Tris, pH 7.5.

Description of Materials

This product contains Dynabeads™ Protein G for immunoprecipitation. Dynabeads™ Protein G beads are uniform, 2.8 µm, superparamagnetic beads with recombinant Protein G (approximately 45 kDa) covalently coupled to the surface.

Related Products

Product	Cat. No.
Immunoprecipitation Kit – Dynabeads™ Protein A	10006D
Immunoprecipitation Kit – Dynabeads™ Protein G	10007D
Dynabeads™ Protein A	10001D
DynaMag™-2	12321D
HulaMixer™ Sample Mixer	15920D
Cell Extraction Buffer	FNN0011
NP40 Cell Lysis Buffer	FNN0021

REF on labels is the symbol for catalog number.

Table 1 Binding strength of Protein G by species and subclass

Ig origin	Affinity for Protein G
Human IgG1,2,4	+++
Human IgD	-
Human IgA, E, M	-
Human IgG3	+++
Mouse IgG1	+++
Mouse IgG2, 2b, 3	+++
Mouse IgM	+
Rat IgG1	+
Rat IgG2a	+++
Rat IgG2b	+
Rat IgG2c	+
Bovine IgG1	+++
Bovine IgG2	+++
Chicken IgY	-
Dog IgG	+
Goat IgG1	+++
Goat IgG2	+++
Guinea Pig IgG	+
Hamster	NA
Horse IgG	+++
Monkey IgG	+++
Porcine IgG	+++
Rabbit IgG	+++
Sheep IgG1	+++
Sheep IgG2	+++

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