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Immunoprecipitation Kit Dynabeads® Protein G

Catalog no. 10007D **Store at 2˚C to 8˚C**

Rev. Date: January 2012 (Rev. 002)

Kit Contents

Kit contents	Volume
Dynabeads® Protein G	2 mL
Ab Binding & Washing Buffer	16 mL
Washing Buffer	28 mL
Elution Buffer	1 mL

Dynabeads® Protein G contains 30 mg Dynabeads®/mL in phosphate buffered saline (PBS), pH 7.4, with 0.01% Tween®-20 and 0.09% sodium azide as a preservative.
Caution: Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

Product Description

This kit is designed for immunoprecipitation of proteins, protein complexes, protein-nucleic acid complexes, and other antigens. Antibody (Ab) is added to the Dynabeads® Protein G. During a short incubation, the Ab binds to the Dynabeads® via their Fc- region. The tube is then placed on a magnet, where the beads migrate to the side of the tube facing the magnet and allow for easy removal of the supernatant.

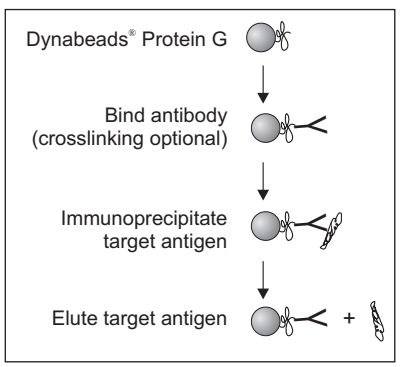

Figure 1: Principle of immunoprecipitation of antigen using Dynabeads® Protein G.

The bead-bound Ab may now be used for immunoprecipitation. Bound material is easily collected utilizing the unique magnetic properties of the Dynabeads®. Magnetic separation facilitates washing, buffer changes, and elution.

Required Materials

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

The following are general recommendations. Alternative buffers may also be used.

- Cell lysis buffer, e.g. Cell Extraction Buffer or NP40 Cell Lysis Buffer
- SDS-PAGE sample buffer, e.g. NuPAGE® LDS Sample Buffer and NuPAGE® Sample Reducing Agent from Invitrogen.

General Guidelines

- Dynabeads® Protein G have a binding capacity of approximately 8 µg human IgG per mg beads. The amount of Ab captured depends on the concentration of Ab and Dynabeads® Protein G in the starting sample (see Table 1).
- For standard immunoprecipitation use PBS for antibody binding and washing steps. However, these may be substituted by other buffers of choice, such as phosphate buffers, lysis buffer (e.g. RIPA, NP40), HEPES, Tris. The recommended elution buffer may also be substituted by alternative low pH-, high pH-, or high salt buffers, depending on your target protein and downstream application.
- Low-affinity antibodies require increased incubation time, thus it can be preferable to pre-incubate sample and antibody prior to bead capture. This improves binding kinetics for the antibody and minimizes non-specific binding. This approach is also recommended when working with protein/nucleic acid complexes, e.g. ChIP.
- An incubation time of only 10 minutes is sufficient for most applications. Increasing the incubation time to 20–120 minutes can increase yield when working with low affinity antibodies, although non-specific binding may increase with increasing incubation times.
- For sensitive proteins and phosphorylation studies, the isolation protocol including elution may be performed at 4°C, to avoid protein complex dissociation and minimize enzymatic activity.

Protocol

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

Prepare Dynabeads®

1. Resuspend Dynabeads® in the vial (vortex >30 sec or tilt and rotate 5 min).
2. Transfer 50 µL (1.5 mg) Dynabeads® 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.

Bind Antibody

1. Add your antibody (Ab) (typically 1–10 µg) diluted in 200 µL PBS with Tween®-20, to the tube from step 4 above. The optimal amount of Ab needed depends upon the individual Ab used.
2. Incubate with rotation for 10 min at room temperature.
3. Place the tube on the magnet and remove the supernatant.
4. Remove the tube from the magnet and resuspend the beads-Ab complex in 200 µL PBS with Tween®-20. Wash by gentle pipetting.

For storage of Ab-conjugated Dynabeads®, use PBS (pH 7.4) with 0.01–0.1% Tween®-20 to prevent aggregation.

Crosslinking

To avoid co-elution of your antibody, crosslink your antibody to the Dynabeads® before continuing with immunoprecipitation. Use the crosslinking reagent BS3. For further information and procedure, visit www.lifetechnologies.com/crosslinking.

Immunoprecipitate Target Antigen

1. Place the tube containing Dynabeads®-Ab complex on the magnet and remove the supernatant.
2. Add your sample containing the antigen (Ag) (typically 100–1,000 µL) and gently pipette to resuspend the Dynabeads®-Ab complex.
3. Incubate with rotation for 10 min at room temperature to allow antigen to bind to the Dynabeads®-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 Dynabeads®-Ab-antigen complex 3 times using 200 µL washing buffer for each wash. Separate on the magnet between each wash, remove supernatant, and resuspend by gentle pipetting.

6. Resuspend the Dynabeads®-Ab-antigen complex in 100 µL washing buffer and transfer the bead suspension to a clean tube. This is recommended to avoid co-elution of proteins bound to the tube wall.

For storage of the immunoprecipitated protein, freeze the Dynabeads®-Ab-Ag complex after adding the elution buffer and sample buffer. For analysis of the sample, thaw and continue with the elution protocol.

Elute Target Antigen

A. Denaturing elution

1. Place the tube containing Dynabeads®-Ab-Ag complex on the magnet and remove the supernatant.
2. Add 20 µL Elution Buffer and 10 µL premixed NuPAGE® LDS Sample Buffer and NuPAGE Sample Reducing Agent (mixed as per manufacturer's instructions).
3. Gently pipette to resuspend the Dynabeads®-Ab-Ag complex.
4. Heat for 10 min at 70ºC.
5. Place the tube on the magnet and load the supernatant/sample onto a gel.

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

B. Non-denaturing elution

1. Place the tube (from step 6 "Immunoprecipitate Target Antigen") on the magnet and remove the supernatant.
2. Add 20 µL elution buffer and gently pipet to resuspend the Dynabeads®- Ab- antigen complex. Avoid foaming.
3. Incubate with rotation for 2 min 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 are uniform, 2.8 µm, superparamagnetic beads with recombinant Protein G (approximately 17 kDa) covalently coupled to the surface.

Related Products

Product	Cat. no.
Immunoprecipitation Kit – Dynabeads® Protein A	10006D
Dynabeads® Protein G	10003D
Dynabeads® Protein A	10001D
DynaMag™-2	12321D
HulaMixer® Sample Mixer	15920D
Cell Extraction Buffer	FNN0011
NuPAGE® LDS Sample Buffer	NP0007
NuPAGE® Sample Reducing Agent	NP0009

REF on labels is the symbol for catalog number.

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Table 1: Binding strength of Protein G to different species of Ig's and their subclasses.

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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