invitrogen

Dynabeads™ Protein A Immunoprecipitation Kit

Catalog No. 10006D **Store at 2˚C to 8˚C**

Publication No. MAN0017347 **Rev.** A.0

Kit contents

Component	Volume
Dynabeads™ Protein A	2 mL
Ab Binding & Washing Buffer	16 mL
Washing Buffer	28 mL
Elution Buffer	1 mL

Dynabeads™ Protein A kit contains sufficient reagents for 40 reactions. The magnetic beads are at a concentration of 30 mg/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.
Note: Read the Safety Data Sheets (SDSs) for additional information on buffers.

Product description

The kit contains Dynabeads™ Protein A beads, as well as buffers for binding, washing, and elution steps for immunoprecipitation of proteins, protein complexes, protein-nucleic acid complexes, and other antigens.

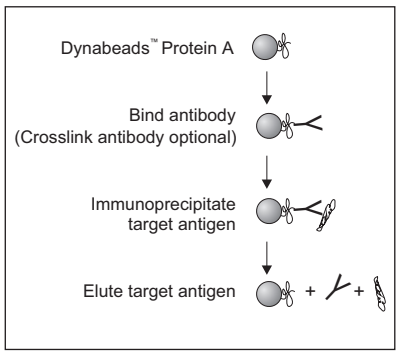

Figure 1: Principle of immunoprecipitation of antigen using Dynabeads™ Protein A.

Antibodies (Ab) are added to the Dynabeads™ Protein A, 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 of the target antigen (Ag). Bound material is easily collected utilizing the unique magnetic properties of the Dynabeads™ magnetic beads.

The Dynabeads™ Protein G kit can also be used as part of an automated workflow on the KingFisher™ platform. Protocols for the KingFisher™ Duo Prime System (up to 24 samples/run) and KingFisher™ Flex™ System (up to 96 samples/run), go to **thermofisher.com/kingfisher**.

Required materials

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

General guidelines

- Dynabeads™ Protein A has 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.
- The choice of primary antibody is the most important factor for successful target Ag capture. Some antibodies exhibit reduced Ag-binding efficiency for immunoprecipitation, even though good results are seen with other immunological assays. The affinity of different antibody types to Protein A is shown in Table 1.
- For low-affinity antibodies or samples with low antigen concentration, pre-incubate the sample and antibody (indirect technique) before 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.
- Due to fast binding kinetics, an antibody only needs to be incubated with the Dynabeads™ magnetic beads for 10 minutes for sufficient antibody capture.
- Increase incubation time of the magnetic bead-Ab complex with the target antigen to 20–120 minutes to increase yield for low affinity antibodies or samples with low antigen concentration, although this may lead to increased non-specific binding.
- For sensitive proteins and phosphorylation studies, perform the isolation protocol and elution at 2–8°C, to avoid protein complex dissociation and minimize enzymatic activity.

Protocol

The protocol is a general guideline for immunoprecipitation. Optimization may be required for each antibody (Ab) and target antigen (Ag). The protocol uses 50 µL of Dynabeads™ Protein A, but this may be scaled up or down as required.

Prepare Dynabeads™ magnetic beads

1. Resuspend Dynabeads™ magnetic beads in the vial (vortex >30 sec or tilt and rotate 5 minutes).
2. Transfer 50 µL (1.5 mg) 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 of Ab Binding and Washing Buffer to the 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 Ab Binding and Washing Buffer. Wash by gentle pipetting.
   Note: Ab-conjugated magnetic beads can be stored in Ab Binding and Washing Buffer to prevent aggregation.
5. Proceed to "Immunoprecipitate target antigen".

(*Optional*) Crosslink antibody

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

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 (typically 100–1,000 µL) and gently pipette to resuspend the magnetic bead-Ab complex.
3. Incubate with rotation for 10 minutes at room temperature to allow the antigen 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 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 magnetic bead-Ab-Ag 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.

   Note: To store the immunoprecipitated protein, add Elution Buffer and NuPAGE™ LDS Sample Buffer, then freeze the magnetic bead-Ab-Ag complex. For subsequent analysis of the sample, thaw and proceed with step 2 of "Elute target antigen (Denaturing elution)".
7. Proceed to "Elute target antigen".

Elute target antigen

Denaturing elution

1. Place the tube containing the magnetic bead-Ab-Ag complex on the magnet and remove the supernatant.
2. Add 20 µL Elution Buffer and 10 µL of pre-mixed NuPAGE™ LDS Sample Buffer and NuPAGE Sample Reducing Agent.
3. Gently pipette to resuspend the magnetic bead-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 magnetic bead-Ab-Ag 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.

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 magnetic bead-Ab-Ag complex. Avoid producing foam during resuspension.
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 A for immunoprecipitation. Dynabeads™ Protein A are uniform, 2.8 µm, superparamagnetic beads with recombinant Protein A (approximately 45 kDa) covalently coupled to the surface.

Related products

Product	Cat. No.
Immunoprecipitation Kit – Dynabeads™ Protein G	10007D
Dynabeads™ Protein G	10003D
Dynabeads™ Protein A	10001D
DynaMag™-2 magnet	12321D
HulaMixer™ Sample Mixer	15920D
Cell Extraction Buffer	FNN0011
NuPAGE™ LDS Sample Buffer	NP0007
NuPAGE™ Sample Reducing Agent	NP0009
DSS (disuccinimidyl suberate)	21655

REF on labels is the symbol for catalog number.

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

Ig origin	Affinity for Protein A
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	+
Horse IgG	+
Monkey IgG	+++
Porcine IgG	+++
Rabbit IgG	+++
Sheep IgG1	+
Sheep IgG2	+++

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