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Immunoprecipitation Kit – Dynabeads® Protein A

For research use only.

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1. PRODUCT DESCRIPTION

This kit contains Dynabeads® Protein A and buffers for immunoprecipitation (see section 1.3 for details).

1.1 Intended Use

This kit is designed for immunoprecipitation of proteins, protein complexes, protein-nucleic acid complexes and other antigens.

1.2 Principle

Antibody (Ab) is added to the Dynabeads Protein A. During a short incubation the Ab will bind to the Dynabeads via their Fc region. The tube is then placed on a Dynal magnet, where the beads will migrate to the side of the tube facing the magnet and allow for easy removal of the supernatant. 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 (Figure 1).

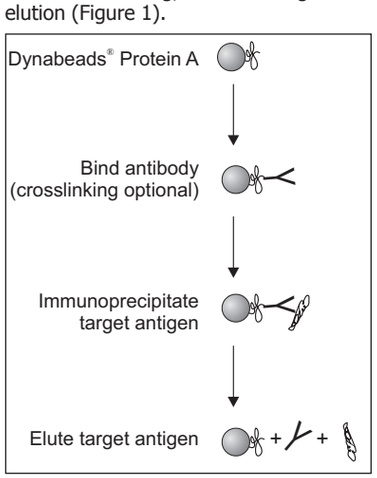

Fig. 1: Principle of immunoprecipitation of target antigen using Dynabeads Protein A.

1.3 Description of Materials

Material supplied

- Dynabeads Protein A (2 ml)
  These uniform, superparamagnetic beads are 2.8 µm in diameter, and have recombinant Protein A (approx. 45 kDa) covalently coupled to their surface. The beads are supplied at a concentration of 30 mg/ml in phosphate buffered saline (PBS), pH 7.4, containing 0.01% Tween®-20 and 0.09% sodium azide (NaN_3).
- Ab Binding & Washing Buffer (16 ml)
- Washing Buffer (28 ml)
- Elution Buffer (1 ml)

All reagents are of analytical grade. The buffers are compatible with protease/phosphatase inhibitors, which may be used if required.

Additional Materials Required

– Magnet: e.g DynaMag™-2 or DynaMag™-Spin (see www.invitrogen.com/magnets for further information and magnet recommendations).
– Mixer allowing rotation of tubes.
– Cell lysis buffer, e.g. Cell Extraction Buffer (Cat. no. FNN0011) or NP40 Cell Lysis Buffer (Cat. no. FNN0021) from Invitrogen
– SDS-PAGE sample buffer, e.g. NuPAGE® LDS Sample Buffer (Cat. no. NP0007) and NuPAGE® Sample Reducing Agent (Cat. no. NP0009) from Invitrogen

1.4 Antibody selection

The choice of primary antibody is the most important factor for successful target antigen capture. Note that some antibodies may show reduced antigen-binding efficiency for immunoprecipitation, even though the antibody shows good results in other immunological assays. Please refer to the manufacturer's recommendations regarding your primary antibody. See also section 3.

2. PROTOCOLS

This protocol offers a general guideline for immunoprecipitation. Optimization may be required for each antibody and target antigen (see also section 3). This protocol format uses 50 µl of Dynabeads Protein A , but may be scaled up or down as required.

2.1 Cell lysis

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, please see www.invitrogen.com/immunoprecipitation.

2.2 Preparation of Dynabeads

1. Completely resuspend Dynabeads by pipetting or by rotating on a roller (5 minutes).
2. Transfer 50 µl (1.5 mg) Dynabeads to a test tube.
3. Separate on the magnet until the supernatant is clear and remove the supernatant.
4. Remove the tube from the magnet
5. Proceed directly to Ab-binding (section 2.3)

2.3 Binding of Antibody

6. Add your antibody (typically 1–10 µg Ab), diluted in 200 µl Ab Binding & Washing Buffer, to the tube from step 4 above. The optimal amount of Ab needed will depend upon the individual Ab used.
7. Incubate with rotation for 10 minutes at room temperature.
8. Place the tube on the magnet and remove the supernatant.
9. Remove the tube from the magnet and wash by gentle pipetting to resuspend the beads in 200 µl Ab Binding & Washing Buffer.
10. Proceed directly to immunoprecipitation (section 2.4).

Crosslinking

Should you wish to crosslink your antibody to the Dynabeads before continuing with immunoprecipitation we recommend use of the cross-linking reagent BS^3 (5 mM) from Thermo Scientific Group (Pierce). For further information and protocol, please visit: www.invitrogen.com/crosslinking.

2.4 Immunoprecipitation of Target Antigen

11. Place the tube (from step 10) on the magnet and remove the supernatant.
12. Add your sample containing the antigen (Ag) (typically 100–1,000 µl) and gently pipette to resuspend the Dynabeads-Ab complex.
13. Incubate with rotation for 10 minutes at room temperature to allow Ag 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. Please see section 3. Technical Advice for details.
14. Place the tube on the magnet. Transfer the supernatant to a clean tube for further analysis, if desired.
15. Wash the Dynabeads-Ab-Ag complex three times using 200 µl Washing Buffer for each wash. Separate on the magnet between each wash, remove supernatant and resuspend by gentle pipetting.
16. Resuspend the Dynabeads-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.
17. Proceed to elution (section 2.5 protocol A or B)

2.5 Elution of Target Antigen

Alternative protocols A or B:

A Denaturing elution

18. Place the tube (from step 17) on the magnet and remove the supernatant.
19. Add 20 µl Elution Buffer and 10 µl premixed NuPAGE LDS Sample Buffer and NuPAGE Sample Reducing Agent (mixed as per manufacturer's instructions). Gently pipette to resuspend the Dynabeads-Ab-Ag complex.
20. Heat for 10 min at 70ºC.
21. Place the tube on the magnet and load the supernatant/sample onto a gel. **Note:** As an alternative, the Dynabeads-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.

B Gentle, non-denaturing elution

18. Place the tube (from step 17) on the magnet and remove the supernatant.
19. Add 20 µl Elution Buffer and gently pipette to resuspend the Dynabeads-Ab-Ag complex. Avoid foaming.
20. Incubate with rotation for 2 minutes at room temperature to dissociate the complex.
21. 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 1M Tris, pH 7.5.

3. TECHNICAL ADVICE

Binding characteristics:

Binding capacity:

Dynabeads Protein A have a binding capacity of approximately 7 µg human IgG per mg beads. The amount of Ab captured depends on the concentration of Ab and Dynabeads Protein A in the starting sample.

Antibody affinity:

Ig origin	Affinity for protein A	Affinity for protein G
Human IgG1,2,4	+++	+++
Human IgD	-	-
Human IgA E, M	+	-
Human IgG3	+	+++
Mouse IgG1	+	+++
Mouse IgG2a,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	+++	+++

Table 1: Binding strength of protein A and G to different species of immunoglobulins (Igs) and their subclasses.

Incubation time

Increasing incubation times during immunoprecipitation can improve yield when working with low affinity antibodies. An incubation time of only 10 minutes is sufficient for most applications. Increasing the incubation time to 20-120 minutes can increase yield. Note that non-specific binding can increase with time.

Incubation temperature

For sensitive proteins, to avoid protein complex dissociation and minimize enzymatic activity e.g. when studying phosphorylation events, the isolation protocol including elution (2.5.B) may be run at 4°C.

Storage of Dynabeads-Ab complex

For storage of Ab-conjugated Dynabeads, we recommend using PBS (pH 7.4) with 0.01-0.1 % Tween-20 to prevent aggregation.

Storage of isolated protein

If the immunoprecipitated protein is to be stored, we recommend freezing the Dynabeads-Ab-Ag complex after adding the Elution Buffer and Sample Buffer (step 2.5.A.19). For analysis of the sample, thaw and continue the elution protocol from step. 2.5.A.20.

Technical Support

Please contact Invitrogen Dynal for further technical information www.invitrogen.com/contact, or visit our website www.invitrogen.com.

4. GENERAL INFORMATION

Invitrogen Dynal AS complies with the Quality System Standards ISO 9001:2000 and ISO 13485:2003.

Storage & Stability

This product is stable until the expiration date stated on the label when stored unopened at 2–8°C. Store Dynabeads vials at 2–8°C. Do not freeze the product. Keep Dynabeads in liquid suspension during storage and all handling steps, as drying will result in reduced performance. Resuspend the beads well before use. The buffers may be stored at room temperature. Use care to avoid bacterial/fungal contamination.

Warnings & Limitations

This product is for research use only. Not intended for any animal or human therapeutic or diagnostic use unless otherwise stated. Preservatives such as sodium azide are toxic if ingested. Avoid pipetting by mouth! Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. When disposing through plumbing drains, flush with large volumes of water to prevent azide build-up. Material Safety Data Sheet (MSDS) is available at http://www.invitrogen.com. Certificate of Analysis/ Compliance is available upon request.

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Related Dynabeads product

Cat. no.	Name
100.07D	Immunoprecipitation Kit - Dynabeads® Protein G
100.01D/02D	Dynabeads® Protein A
100.03D/04D	Dynabeads® Protein G

A comprehensive range of Dynabeads for use in proteomic workflows are available. Please visit www.invitrogen.com.

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