invitrogen™ | DYNAL® invitrogen bead separations

Cat. no. 100.01D
100.02D

Rev. no. 005

Dynabeads® Protein A

For research use only

INDEX

1. PRODUCT DESCRIPTION

Dynabeads Protein A are uniform, 2.8 µm, superparamagnetic beads with Protein A covalently coupled to the surface. The protein A employed is a recombinant *Staphylococcus* protein A lacking the albumin binding region. The molecular weight is 46 kDa.

1.1 Intended Use

Dynabeads® Protein A are designed to capture immunoglobulins (Igs) for immunoprecipitation of proteins, protein complexes, protein-nucleic acid complexes and other antigens.

1.2 Principle

Igs are added to pre-washed Dynabeads Protein A. During a short incubation the Igs will bind to the Dynabeads Protein A via their Fc part. The tube is then placed on a Dynal magnet, where the beads will be immobilized at the side of the test tube and allow for easy removal of the supernatant. The bead-bound Igs may now be used for immunoprecipitation (Figure 1).

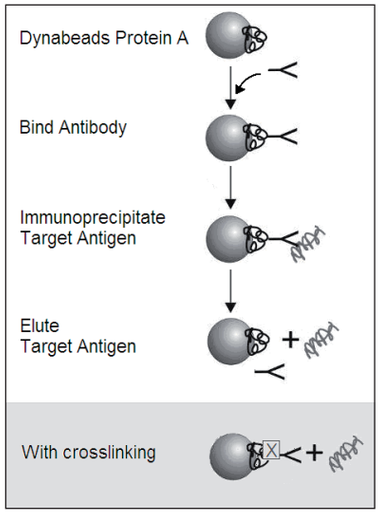

Figure 1: Principle of the use of Dynabeads Protein A for capture of Igs for immunoprecipitation of target antigen.

1.3 Description of Materials

Material supplied

Dynabeads Protein A are supplied in phosphate buffered saline (PBS), pH 7.4, containing 0.1% Tween®-20 and 0.02% sodium azide (NaN_3).

Cat. no. 100.01D: 1 ml
Cat. no. 100.02D: 5 ml

Additional Materials Required

– Magnet: see www.invitrogen.com/magnets-selection for magnet recommendations.
– Mixer: Allowing tilting and rotation of tubes.
– Buffers and reagents:

Wash and Bind buffer (W&B buffer)	0.1 M NaPhospate 0.01 % Tween 20 pH 8.2
Washing buffer	PBS **or** Lysis buffer, e.g. NP40/RIPA
Elution buffer	**Gentle conditions:** 50 mM Glycine pH 2.5-3.0 **and** 1.0 M Tris pH 7.5 **Denaturing conditions:** NuPAGE LDS Sample Buffer (Cat.no NP0007) **and** Reducing Agent (Cat no. NP0004)

For information on using other buffers se **www.invitrogen.com/immunoprecipitation** under Related info.

1.4 Antibody selection

The choice of primary antibody is the most important factor for successful antigen capture. Note that some antibodies may show reduced antigen-binding efficiency for IP even though the antibody shows good results in other immunological assays.
Please refer to the producers recommendations regarding your primary antibody.

2. IMMUNOPRECIPITATION PROTOCOL

This protocol offers a general guideline for immunoprecipitation. Optimization may be required for each antibody and target antigen.

This protocol is an example describing immunoprecipitation using 50 µl of Dynabeads Protein A and may be scaled up and down as required. For further details, please consult our website at: **www.invitrogen.com/immunoprecipitation**

2.1 Washing/Preparation of Dynabeads

1. **Resuspend Dynabeads** by vortexing for 20 seconds.
2. **Transfer 50 µl Dynabeads** to a test tube.
3. Separate on magnet for 1 min and **remove the supernatant.**
4. Remove the tube from the magnet and **add 200 µl W&B buffer.**
5. **Repeat step 3 and 4.**

2.2 Ig coupling to Dynabeads Protein A

1. **Dilute** approximately 5 µg **Antibody in 200 µl W&B buffer.**
2. Place the tube containing **Dynabeads Protein A** from step 2.1.5 on the magnet, separate and **remove the supernatant.**
3. **Add the diluted antibody** to the beads, and resuspend.
4. **Incubate for 10 minutes** with rotation at room temperature.
5. Place the tube on the magnet and **remove the supernatant.**
6. Remove the tube from the magnet and **add 200 µl washing buffer**. Wash the Dynabeads by repeating steps 5 and 6 twice.
7. Place the test tube on the magnet and remove the supernatant.

Crosslinking
You may wish to crosslink your antibody to the Protein A Dynabeads before continuing with the IP protocol. We recommend the cross-linker BS3, 5 mM from Thermo Scientific Group (Pierce) Please consult our website for further information and protocol at:
www.invitrogen.com/immunoprecipitation

2.3 Target Antigen capture

1. **Resuspend the Dynabeads-Ig complex using your sample containing the target antigen** (10-1000 µl cell lysate)
2. **Incubate for 10 minutes** with rotation at room temperature.
3. Place the tube on the magnet and **remove the supernatant**.
4. **Wash** the Dynabeads-Ig-Antigen complex **3 times using 200 µl washing buffer each time**. Resuspend beads in 100 µl washing buffer and **transfer to a clean tube**. NOTE: As some proteins bind unspecifically to the tube wall it is important to transfer the bead-bound complex to a fresh tube to avoid coelution of these unspecific proteins.

2.4 Elution

Alternative protocols A and B

A Denaturing elution

1. Place the tube on the magnet and **remove the supernatant**.
2. **Resuspend the Dynabeads-Ig-Antigen complex in 20 µl of 1x NuPAGE LDS Sample Buffer.**
3. **Heat for 10 min at 70ºC.** The complex will dissociate when heated. Place the tube on a magnet before applying the sample to the gel.

B Gentle, non-denaturing elution

1. Place the tube on the magnet and **remove the supernatant.**
2. **Add 20 µl of 50 mM Glycine, pH 2.8 to the Dynabeads-Ig-Antigen complex.**
3. **Resuspend the complex** gently by pipetting up and down a few times. Avoid foaming.
4. **Incubate with rotation for 2 minutes** at room temperature to dissociate the complex.
5. Place the tube on the magnet and **transfer the supernatant containing eluted Ig and target antigen to a clean tube.**
6. **Adjust the pH** of the eluate by adding an equal volume of 1M Tris, pH 7.5.

3. TECHNICAL ADVICE

Storage of Ig- conjugated Dynabeads
Add 0.01-0.1 % Tween-20 to the storage buffer to prevent aggregation of the beads with immobilized Ig or protein.

Binding characteristic

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.

Binding capacity
The amount of Igs captured depends on the concentration of Igs and Dynabeads Protein A in the starting sample.

Incubation time
Increasing incubation times can improve yield when working with low affinity antibodies or if you dilute original bead volume more than 5 times. Note that nonspecific binding will usually increase with time.

For most applications an incubation time of only 10 min is sufficient, but for some antibodies increasing incubation time to 30 min can maximize binding.

pH
The optimal pH for binding of Igs to Dynabeads Protein A may vary for different Igs, however a pH of around 8 is suitable for most Igs.

Working temperature
For sensitive proteins or protein complexes, the protocol may be ran at 4°C.

Technical Support
Please contact Invitrogen Dynal for further technical information (see contact details). 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 opened vials at 2-8°C and use care to avoid bacterial contamination. Do not freeze the product. Keep Dynabeads in liquid suspension during storage and all handling steps, as drying will result in reduced performance. Resuspend well before use.

Warnings and 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 buildup. 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. 100.03D/04D Dynabeads® Protein G

5. REFERENCES:

Co-immunoprecipitation:
Lin-Lee Y-C et al. Nuclear localization in the biology of the CD40 receptor in normal and neoplastic human B lymphocytes. J.Biol.Chem. 2006;281: 18878- 18887

Haren L et al. NEDD1-dependent recruitment of the gamma-tubulin ring complex to the centrosome is necessary for centriole duplication and spindle assembly. J.Cell Biol. 2006;172:505-515

Pham LV et al. Constitutive NF-kappaB and NFAT activation in aggressive B-cell lymphomas synergistically activates the CD154 gene and maintains lymphoma cell survival. Blood 2005;106(12):3940- 3947

Catrein I et al. Experimental proof for a signal peptidase I like activity in Mycoplasma pneumoniae, but absence of a gene encoding a conserved bacterial type I SPase. FEBS J. 2005;272:2892- 2900

Purification of IgG from sera:
Kudva IT et al. Identification of a protein subset of the Anthrax spore immunome in humans immunized with the Anthrax vaccine adsorbed preparation. Infect. Immun.2005;73:5685-5696

Chromatin immunoprecipitation:
Wu Z et al. Interleukin-21 receptor gene induction in human T cells Is mediated by T-cell receptor-induced Sp1 activity. Mol. Cell.Biol. 2005;25:9741- 9752

Co-immunoprecipitation, automatic washing:
Wan L et al. The survival of motor neurons protein determines the capacity for snRNP assembly: Biochemical deficiency in spinal muscular atrophy. Mol. Cell.Biol. 2005;25(13):5543-5551

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