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Cat. no. 100.01D
100.02D

Rev. no. 004

Dynabeads® Protein A

For research use only.

INDEX

1. PRODUCT DESCRIPTION
   1.1 Intended Use
   1.2 Principle
   1.3 Description of Material
2. PROTOCOLS
   2.1 Washing Procedure
   2.2 Ig Capture Procedure
   2.3 Ig Elution Procedure
   2.4 Immunoprecipitation
   2.5 Re-use of Dynabeads Protein A
3. TECHNICAL ADVICE
4. GENERAL INFORMATION

1. PRODUCT DESCRIPTION

1.1 Intended Use

Dynabeads® Protein A is designed to capture immunoglobulins (Ig) for small scale purification purposes or for downstream immunoprecipitation of proteins or other antigens (Figure 1).

1.2 Principle

Immunomagnetic protein isolation using Dynabeads Protein A provides a fast and reliable method for capturing Ig for small scale purification or downstream immunoprecipitation. Ig can be isolated directly from acites, serum, tissue culture supernatants or other samples.

An Ig-containing sample is added to a tube containing pre-washed Dynabeads Protein A. During a short incubation, the immunoglobulins will bind to Dynabeads Protein A via their Fc part. Place the test tube on a magnet (Dynal MPC™) to collect the Dynabeads Protein A - Ig complex at the tube wall, and discard the supernatant.

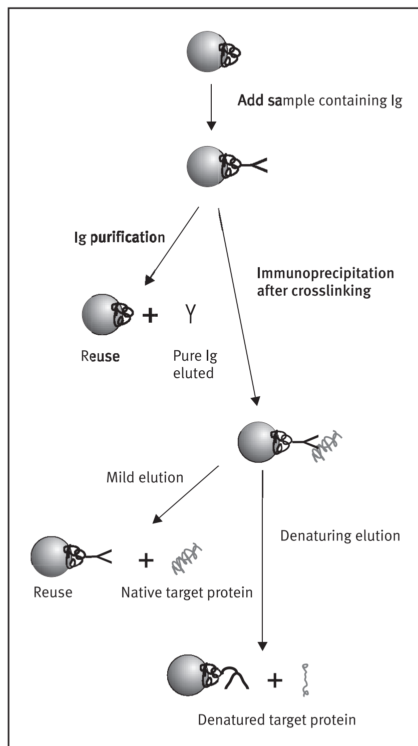

Figure 1: Principle of the use of Dynabeads Protein A for small scale purification of Ig's and for downstream immunoprecipitation of target antigen.

The purified and concentrated Ig can be eluted off in a small volume for downstream use such as antibody labelling or epitope mapping.

The Dynabeads Protein A - Ig complex can also be used directly to immunoprecipitate a target antigen, or for immunodepletion (for references, see section 3.2 below). Add the Dynabeads Protein A - Ig complex directly to a sample (cell lysate or other) containing your target antigen and incubate for antibody-antigen complex formation. Place the test tube on a magnet to collect the complex at the tube wall, and discard the supernatant. Resuspend the beads in a small volume for further use, or elute off your target protein directly e.g. in an acidic buffer or boil in a small volume of SDS-PAGE application buffer.

If your downstream application involves purification of your target protein, you might want to cross-link the Ig to the protein A on the Dynabeads before immunoprecipitation to prevent co-elution of the Ig. This is not necessary for downstream SDS-PAGE followed by autoradiography or Western blotting, and optional for Silver or Coomassie staining.

1.3 Description of Material

Dynabeads Protein A are uniform, magnetizable polystyrene beads covalently coupled with recombinant protein A.

Typical characteristics for any given lot of this product:

Diameter: 2.8 µm ± 0.2 µm (C.V.max 3%)
Density: approx. 1.3 g/cm^3
Surface area: 3-9 m^2/10^9 Dynabeads

The beads 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

Protein A

Protein A has a high specificity for immunoglobulins (Table 1) and is therefore suitable for the one-step capture of Ig (1). The native bacterial cell wall protein is a single polypeptide chain of 42 kDa with four Ig Fc binding sites, two of which are active (2). The protein A employed in this product is a 45 kDa recombinant protein containing all four binding sites for the Fc region of Ig, but without any albumin binding sites.

Binding capacity

Binding of Ig to protein A in solution is an equilibrium reaction. In order to capture as many Ig as possible, it is important to keep the reaction volume low to maintain high concentrations of beads and Ig. There is no need to pre-treat or dilute the sample (even viscous samples). For both the immobilisation of Ig and downstream immunoprecipitation procedures, it is recommended to keep the concentration of beads in the sample close to its original concentration in the Dynabeads Protein A vial. The binding efficiency will decrease if the Dynabeads are suspended in more than 5 times the original volume of Dynabeads initially added.

The amount of Ig captured is dependent on the concentration of Ig in the starting sample. 100 µl Dynabeads Protein A will isolate approximately 25-30 µg human IgG from a sample containing 20-200 µg IgG/ml. A higher volume of Dynabeads is recommended to avoid waste of Ig when working with concentrated samples or the Ig is precious. Keep all other parameters fixed as described below. Maximum amount of Ig-binding is obtained after 10 minutes (Figure 2).

Ig origin	Protein A
Human IgG1,2,4	Strong
Human IgD	No binding
Human IgG3,A,E,M	Weak
Mouse IgG1	Weak
Mouse IgG2a,2b, 3	Strong
Mouse IgM	Weak
Rat IgG1	Weak
Rat IgG2a	No binding
Rat IgG2b	No binding
Rat IgG2c	Strong
Bovine IgG1	Weak
Bovine IgG2	Strong
Chicken IgY	No binding
Dog IgG	Strong
Goat IgG1	Weak
Goat IgG2	Strong
Guinea pig IgG	Strong
Hamster	Weak
Horse IgG	Weak
Monkey IgG	Strong
Porcine IgG	Strong
Rabbit IgG	Strong
Sheep IgG1	Weak
Sheep IgG2	Strong

Table 1: Binding strength of protein A to different species of immunoglobulins (Ig) and their subclasses. Monoclonal antibodies will vary in their affinity towards protein A.

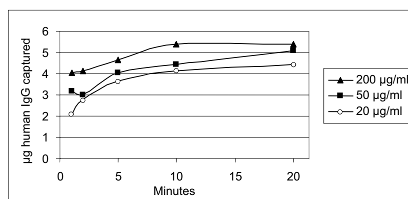

Figure 2: Rapid binding kinetics of Dynabeads Protein A (14 µl) for capture of human IgG in a 100 ml sample.

2. INSTRUCTIONS FOR USE

Dynabeads Protein A should be washed prior to use. Washed Dynabeads Protein A are resuspended in a basic phosphate buffer (e.g. pH 8.1) to facilitate binding of Ig to beads. The pH in the sample containing Ig might be adjusted for the same reason using a basic 5 x stock solution.

The procedure described below is for the isolation of approximately 25 µg IgG from 10 µl human serum (or similar sample). The use of polypropylen tubes is recommended for washing and Ig capture.

2.1 Washing Procedure

The washing procedure is facilitated by the use of a magnet (Dynal MPC).

1. Resuspend the Dynabeads Protein A, thoroughly in the vial (e.g. by vortexing 1-2 minutes or rotating on a roller) to obtain a homogeneous suspension.
2. Transfer 100 µl Dynabeads Protein A to a test tube at room temperature. (Please refer to section 1.3 above for details on binding capacity.)
3. Place the test tube on the magnet for one minute and pipette off the supernatant.
4. Remove the test tube from the magnet and add 0.5 ml 0.1 M Na-phosphate buffer pH 8.
5. Repeat steps 3, 4 and 3.

2.2 Ig Capture Procedure

In this example, the Dynabeads volume is much larger than the sample volume. In cases where the sample volume is as large as 1/4 the Dynabeads volume, add 0.5 M Na-phosphate 5 x stock solution to raise the pH in the sample to 8 (with a final molarity of 0.1) before adding to the Dynabeads (i.e. ad 10 µl stock solution to each 40 µl sample).

Dynabeads are resuspended in an adjusted volume so that sample and Dynabeads volumes together is the same as the bead-volume originally pipetted from the vial.

1. Resuspend the washed Dynabeads in 90 µl 0.1 M Na-phosphate buffer pH 8.
2. Add 10 µl serum to the solution containing Dynabeads.
3. Incubate with slow tilt rotation mixing for 10 minutes at room temperature.
4. Place the test tube on the magnet for 2 minutes and pipette off the supernatant.
5. Remove the test tube from the magnet and add 0.5 ml 0.1 M Na-phosphate buffer pH 8. (For downstream immunoprecipitation or storage of Dynabeads, 0.01-0.1% Tween-20 can be added to the buffer to prevent aggregation of the Dynabeads Protein A - Ig complex.)
6. Repeat steps 4, 5, 4, 5, 4.

The purified Ig is now ready to be eluted off the Dynabeads (see 2.3 below) or the Dynabeads Protein A - Ig complex can be used for immunoprecipitation - either by adding directly to a new sample containing the target protein, or by first cross-linking the Ig covalently to the protein A on the Dynabeads (see 2.4 below).

2.3 Ig Elution Procedure

Eluting Ig off the Dynabeads Protein A is, in this example, performed by lowering pH using 0.1 M citrate (pH 2-3) as the elution buffer. The degree of acidity needed depends on the species and Ig subclass, but at pH 3 most Ig will be eluted.

1. Add an appropriate amount (e.g. 40 µl) 0.1 M citrate (pH 2-3) to the Dynabeads Protein A - Ig complex with immobilised IgG.
2. Mix well by tilting and rotation 2 minutes.
3. Place the test tube on a magnet and transfer the supernatant, containing purified Ig, to a clean tube.

Immediately adjust the eluate to physiologic pH by adding alkaline buffer (e.g. 1M Tris pH 7.5-9).

2.4 Immunoprecipitation

When isolating antigens for SDS-PAGE followed by Western blotting or autoradiography the presence of Ig will not disturb your detection system. For other applications (e.g. protein purification, amino acid sequencing or when the Dynabeads Protein A with bound Ig is to be reused) co-elution of the Ig is not desired. To prevent this, the captured Ig can be crosslinked to the protein A on the Dynabeads. Cross-linking is also necessary if the Dynabeads - Ig complex is reused for immunoprecipitation.

2.4.1 Crosslinking

The protocol presented below is an example using one of several commercially available cross-linkers.

1. Add 1 ml 0.2 M triethanolamine, pH 8.2 to the Dynabeads - Ig complex with immobilised immunoglobulin. Wash twice according to procedure 2.1 above, using 0.2 M triethanolamine, pH 8.2 as the washing buffer.
2. Resuspend the Dynabeads - Ig complex in 1 ml of 20 mM DMP (dimetyl pimelimidate dihydrochloride, Pierce #21666) in 0.2 M triethanolamine, pH 8.2 (5.4 mg DMP/ml buffer). This cross-linking solution must be prepared immediately before adding to the Dynabeads - Ig complex.
3. Incubate with rotational mixing for 30 minutes

at 20°C. Place the tube on the magnet and discard the supernatant.

4. Remove the tube from the magnet and stop the reaction by resuspending the Dynabeads - Ig complex in 1 ml of 50 mM Tris, pH 7.5 and incubate for 15 minutes with rotational mixing.
5. Place the tube on the magnet and discard the supernatant.
6. Wash the now crosslinked Dynabeads - Ig complex 3 times with 1 ml PBS pH 7.4 by the use of a magnet, according to procedure 2.1 above. Resuspend the Dynabeads - Ig complex to 100 µl or add directly to antigen-containing solution. The full recovery of your Ig activity cannot be guaranteed, as this varies from Ig to Ig.

NOTE:

The protocol presented here uses 0.2 M triethanolamine pH 8.2. Other non-amine containing buffers with pH 7-9 can also be used (see e.g. http://www.piercenet.com/Products/Browse.cfm?fldID=020302).

2.4.2 Binding of Antigen

Trace amounts of Ig not cross-linked to Dynabeads Protein A can be removed prior to binding by following the elution procedure described in 2.3 above. Binding of protein or other antigen to the Dynabeads - Ig complex is dependent on the concentration of the Dynabeads, antigen concentration, the affinity of the immobilised Ig and incubation time. Binding is performed at 2-8°C from 10 minutes to 1 hour.

Equilibrium antibody-antigen is reached at approximately 1 hour.

1. Add sample containing antigen to the Dynabeads - Ig complex. For a 100 kD protein, use a volume containing approximate 25 µg target antigen per ml of beads to assure an excess of antigen. If dilution of antigen is necessary, PBS or 0.1 M phosphate buffer (pH 8) can be used as dilution buffer.
2. Incubate with tilting and rotation for one hour. (Incubation times as low as 10 minutes can be used with concentrated protein samples in volumes close to what was originally pipetted from the vial).
3. Place the tube on the magnet for 2 minutes to collect the Dynabeads - Ig complex at the tube wall. For viscous samples, double the time on the magnet. Pipette off the supernatant.
4. Wash the Dynabeads - Ig complex 3 times using 1 ml PBS each time and change buffers by the use of a magnet, according to procedure 2.1 above.

2.4.3 Target Protein Elution Procedure

Conventional elution methods can be applied for the elution of target antigen from the Dynabeads - Ig complex. Low pH (2-3), change in ionic strength, affinity elution, electrophoresis, polarity reducing agents, deforming eluents can be applied, or even boiling the beads in SDS-PAGE application buffer for direct characterisation of protein on SDS-PAGE. The method of choice depends on the Ig's affinity for the antigen, stability of target protein and downstream applications and detection methods. Most antigens will be eluted at pH 3 following the procedure described under 2.3 above. If the Dynabeads - Ig complex is to be reused, mild elution methods should be employed. To prevent aggregation of the beads with immobilised Ig, 0.01-0.1% Tween-20 can be added to the storage buffer.

2.5 Re-use of Dynabeads Protein A

For re-use after elution, the Dynabeads Protein A should be brought to neutral pH using a Na-phosphate buffer, pH 7.

3. TECHNICAL ADVICE

For further technical information, please visit www.invitrogen.com/DynabeadsProteinAG, or contact Invitrogen Dynal for further technical support.

3.1 Additional Material Required

- Magnet: Dynal MPC™ e.g. Dynal MPC™-S (Cat. no. 120.20D) for 20 µl to 2 ml samples
- Mixer: Allowing tilting and rotation of tubes e.g. Dynal MX1 (Cat. no. 159.07) or Dynal Sample Mixer (Cat. no. 947.01)
- Buffers and reagents

3.2 References

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4. GENERAL INFORMATION

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

4.1 Storage and 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.

4.2 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.

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The products are warranted to the original purchaser only to conform to the quantity and contents stated on the vial and outer labels for the duration of the stated shelf life. Invitrogen Dynal's obligation and the purchaser's exclusive remedy under this warranty is limited either to replacement, at Invitrogen Dynal's expense, of any products which shall be defective in manufacture, and which shall be returned to Invitrogen Dynal, transportation prepaid, or at Invitrogen Dynal's option, refund of the purchase price.

Claims for merchandise damaged in transit must be submitted to the carrier.

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