novex®
by life technologies™

Dynabeads® M-280 Sheep anti-Mouse IgG

Catalog nos. 11201D, 11202D

Dynabeads® M-280 Sheep anti-Rabbit IgG

Catalog nos. 11203D, 11204D

Store at 2˚C to 8˚C

Rev. Date: May 2012 (Rev. 000)

Product Contents

Product contents	Cat. no.	Volume
Dynabeads® M-280 Sheep anti-Mouse IgG	11201D	2 mL
	11202D	10 mL
Dynabeads® M-280 Sheep anti-Rabbit IgG	11203D	2 mL
	11204D	10 mL

Dynabeads® M-280 Sheep anti-Mouse IgG and Dynabeads® M-280 Sheep anti-Rabbit IgG both contains 6–7 × 10^8 beads/mL (~10 mg/mL) in phosphate buffered saline (PBS) pH 7.4 with 0.1% bovine serum albumin (BSA) and 0.02% sodium azide.
Caution: Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

Product Description

Note that this manual describes the protocols for two separate products: Dynabeads® M-280 Sheep anti-Mouse IgG and Dynabeads® M-280 Sheep anti-Rabbit IgG. The protocols and handling steps are the same; the only difference being different primary antibody targets:

- Dynabeads® Sheep anti-mouse IgG bind defined antigens via a mouse primary antibody.
- Dynabeads® Sheep anti-Rabbit IgG bind defined antigens via a rabbit primary antibody.

Dynabeads® M-280 Sheep anti-Mouse IgG and Dynabeads® M-280 Sheep anti-Rabbit IgG are designed as a solid support for simple and efficient binding of immunoglobulins (Ig) or other target molecules (see fig. 1).

The size of the beads (2.8 µm) makes them particularly suitable for isolation of antibodies (Ab) and their target proteins. The beads can also be used for cell isolation, but visit www.lifetechnologies.com/dynabeads to view our full range of cell isolation products.

The beads with primary antibody may be added directly to the sample containing your target antibody/antigen. The beads bind to the target during a short incubation, then the bead-bound target is separated by a magnet (direct technique). Alternatively, the primary antibody is allowed to bind to the target in suspension prior to adding the beads (see fig. 1).

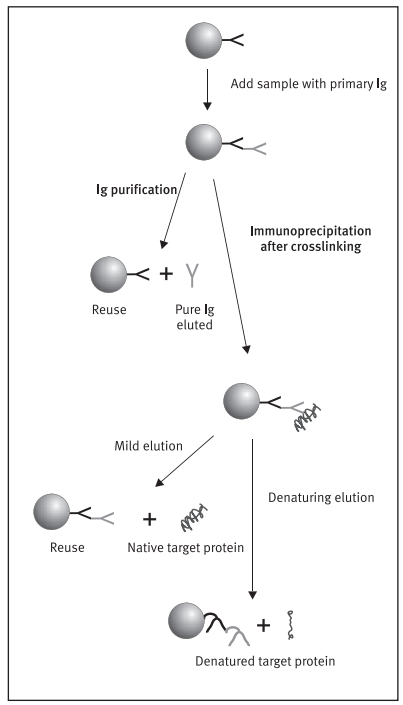

Figure 1: Overview of method

Required Materials

- Magnet (DynaMag™ portfolio). See www.lifetechnologies.com/magnets for recommendations.
- Mixer allowing tilting and rotation of tubes (e.g. HulaMixer® Sample Mixer).
- Primary mouse antibody for Dynabeads® M-280 Sheep anti-Mouse IgG.
- Primary rabbit antibody for Dynabeads® M-280 Sheep anti-Rabbit IgG.

Recommended Buffers

- Washing Buffer: Ca^{2+} and Mg^{2+} free phosphate buffered saline (PBS) supplemented with 0.1% bovine serum albumin (BSA) and 2 mM EDTA, pH 7.4.
  Note: BSA can be replaced by human serum albumin (HSA) or fetal calf serum (FCS). EDTA can be replaced by sodium citrate.
- Elution Buffer: 0.1 M citrate, pH 2-3.

General Guidelines

- Visit www.lifetechnologies.com/samplepreparation for recommended sample preparation procedures.
- Use a mixer that provides tilting and rotation of the tubes to ensure that the beads do not settle in the tube.
- These products should not be used with the MPC™-1 magnet (Cat. no. 12001D).
- Avoid air bubbles (foaming) during pipetting.
- Never use less than recommended volume of beads.
- Carefully follow the recommended volumes and incubation times.

Direct vs. Indirect Technique

- *Use the indirect technique when:* The concentration of antibody is low, the antibody-antigen affinity is weak, the binding kinetics is slow or the direct technique gives unsatisfactory purity.
- *Use the direct technique when:* The affinity of the primary antibody is high, there are high numbers of target antigens, or to make a larger stock preparation of primary coated beads (will generally have the same shelf life as stated on the beads vial).

Cross-linking prior to Immunoprecipitation

Immunoprecipitation (IP) is done by either adding the primary coated beads directly to a new sample containing the target protein, or by first covalently cross-linking the primary Ig to the antibody on the beads. Bound Ig will be co-eluted along with the target using different elution methods (e.g. for SDS-PAGE followed by Western blotting or autoradiography). For other applications (e.g. protein purification or amino acid sequencing) where co-elution of the Ig is not desired, the primary Ig should be cross-linked to the antibody on the beads prior to IP. If the Ig-coated beads are to be re-used, cross-linking is necessary.

Target Protein Elution

One of the major advantages of using Dynabeads® products in protein/Ig isolation is the possibility to elute in small volumes. Low pH (2.8–3.5), change in ionic strength, affinity elution, electrophoresis, polarity reducing agents, deforming eluents can be applied, or even boiling the bead-target complex in SDS-PAGE application buffer for direct characterization of protein on SDS-PAGE. The method of choice depends on the Ig's affinity for the specific target protein, stability of target protein and downstream applications and detection methods. Most proteins will be eluted at pH 3.1. Some protein functionality might be lost under these conditions. If maintaining functionality of the target protein is important, try milder elution conditions, such as high salt (e.g. 2 M NaCl) or step-wise elution reducing pH from 6 down to 3. This is also recommended if the bead-bound ligand must remain functional to allow re-use of the beads.

Protocol

Wash the Beads

See "Couple Beads with Target Ig" to determine the bead volume.

1. Resuspend the beads in the vial (i.e. vortex for >30 sec, or tilt and rotate for 5 min).
2. Transfer the desired volume of beads to a tube.
3. Add the same volume of Washing Buffer, or at least 1 mL, and resuspend.
4. Place the tube in a magnet for 1 min and discard the supernatant.
5. Remove the tube from the magnet and resuspend the washed beads in the same volume of Washing Buffer as the initial volume of beads (step 2).

Couple Beads with Target Ig

- This protocol is based on 50 µL Dynabeads® Sheep anti-Mouse IgG or Dynabeads® Sheep anti-Rabbit IgG, but is directly scalable. It is not recommended to work with lower volumes. When working with larger volumes, scale up all volumes accordingly.
- Use 0.4–4 µg Ig/50 µL beads. Optimize for your application.

6. Add the sample containing ~0.4–4 µg target-Ig (optimize) to 50 µL of pre-washed and resuspended beads.
7. Incubate with gentle tilting and rotation for 30 min or up to 24 hours at 2°C to 8°C.
8. Place the test tube on the magnet for 2 min and pipet off the supernatant.
9. Remove the test tube from the magnet; add 1 mL Washing Buffer and resuspend.
10. Repeat steps 4 and 5 twice.
11. Place the tube on the magnet and remove the supernatant.

Elute Isolated Ig

- Elute the isolated Ig off the beads using 0.1 M citrate (pH 23) to lower the pH. Most Ig will be eluted off at pH 3.1, but the degree of acidity required will depend on the specific Ig.
- This protocol is based on the 50 µL beads from the "Couple the Beads with Target Ig" section. If using higher volumes, scale up accordingly.

1. Add 50 µL 0.1 M citrate to the Ig-coupled beads.
2. Mix well by tilting and rotation for 2 min.
3. Place the test tube on a magn}et for 2 min and transfer the supernatant containing the purified Ig to a new tube.
4. Add another 50 µL 0.1 M citrate to the bead fraction to elute any remaining Ig.
5. Mix well by tilting and rotation for 2 min.
6. Place the test tube on a magnet for 2 min, pipet off the eluate and pool the two supernatants containing pure Ig.

The Ig-eluted beads may be re-used at least five times. For re-use after elution, the beads should immediately be brought to neutral pH using a Na-phosphate buffer. For storage, the beads should be resuspended in Washing Buffer.

Cross-linking Ig to the Beads

If you want to avoid co-elution of the antibody, cross-link your antibody to the beads before continuing with e.g. IP. We recommend using the cross-linking reagent BS3.For further information and procedure, visit: www.lifetechnologies.com/crosslinking.

Antigen-Binding to Ig-Coated Beads

Use approximately 25 µg target antigen/mL beads to assure an excess of antigen. Dilute with PBS or 0.1 M phosphate buffer (pH 7–8), if necessary.

1. Add 25 µg target antigen/mL Ig-coupled beads.
2. Mix well by tilting and rotation for 1 hour. (Incubation time can be reduced to as low as 10 min, if the protein concentration is high).
3. Place the tube on the magnet for 2 min and pipet off the supernatant.
4. Remove the test tube from the magnet; add 1 mL Washing Buffer and resuspend.
5. Repeat steps 3–4 twice.

See "General Guidelines" for more information on elution of the target protein.

Description of Materials

Dynabeads® M-280 Sheep anti-Mouse IgG are uniform, superparamagnetic, polystyrene beads with affinity purified sheep anti-mouse IgG covalently bound to the bead surface. The antibody binds both heavy and light chains of mouse IgG1, IgG2a and IgG2b. They may have low reactivity towards mouse IgG3 and IgM. Human cross reactivity is minimal.

Dynabeads® M-280 Sheep anti-Rabbit IgG are uniform, superparamagnetic, polystyrene beads with affinity purified sheep anti-rabbit IgG covalently bound to the bead surface. The antibody binds all rabbit IgG subclasses.

Related Products

Product	Cat. no.
DynaMag™-2	12321D
DynaMag™-5	12303D
DynaMag™-15	12301D
Dynabeads® Protein A	10001D
Dynabeads® Protein G	10003D
HulaMixer® Sample Mixer	15920D

REF on labels is the symbol for catalog number.

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