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Dynabeads® M-270 Epoxy

Catalog nos. 14301, 14302D **Store at 2˚C to 8˚C**

Rev. Date: June 2012 (Rev. 005)

Product Contents

Cat. no.	Volume
14301	60 mg
14302D	300 mg

Dynabeads® M-270 Epoxy contains ~6.7 × 10^7 beads/mg, supplied as freeze-dried powder.

Product Description

Dynabeads® M-270 Epoxy is used as a solid support in a wide variety of biomagnetic separations. The hydrophilic surface ensures low non-specific binding, excellent dispersion abilities and easy handling of the beads in a wide variety of buffers. The size makes them particularly suitable for protein isolation for sample preparation, bioassays, selection of affinity binders, etc.

Via primary amino or sulfhydryl groups in the ligand the beads can be coated directly with proteins, peptides, antibodies, enzymes or other target specific molecules for isolation of e.g. hormones, receptors, disease markers, or bacteriophages.

Once coupled with ligand, the beads are added to the sample containing your target molecule. After a short incubation allowing affinity capture of the target by the beads, the beads are applied to a magnet. The unwanted supernatant can be removed and the beads are washed to give a pure sample. Bead-bound targets can be used directly in bioassays, boiled in application buffer and analyzed on SDS-PAGE, or elute off the beads with conventional elution methods.

Note: If you want a total antibody coupling solution, use Dynabeads® Antibody Coupling Kit that includes both Dynabeads® M-270 Epoxy and the required buffers.

Required Materials

- Magnet (DynaMag™ portfolio). See www.lifetechnologies.com/magnets for recommendations.
- Mixer allowing tilting and rotation of tubes (e.g. HulaMixer® Sample Mixer).
- Buffers/solutions: See "Recommended buffers/solutions".
- Antibody/other selecting molecule.

Recommended Buffers/Solutions

Other buffers might be used, but buffers containing amino groups (e.g. Tris) should not be used for coating of ligand.

General Guidelines

- Use a mixer that provides tilting and rotation of the tubes to ensure that beads® do not settle in the tube.
- Avoid air bubbles (foaming) during pipetting.
- Carefully follow the recommended volumes and incubation times.

Ligand Coating Optimalization

Use >3 µg pure ligand per 10^7 Dynabeads® M-270 Epoxy for coupling of small ligands (such as peptides), and slightly higher concentrations for coupling of larger ligands, and a concentration of 1–2 × 10^9 beads/mL during incubation. To enhance binding, use a final concentration of 1–3 M sulfate during coating. The optimal ammonium sulfate concentration depends on the nature of the ligand. A hydrophilic or small ligand (peptide) requires high ammonium sulfate concentration (up to 3 M), whereas most proteins will be sufficiently coated with 1 M ammonium sulfate. Some biomolecules may even lose their function (some Igs) at molarities >1.5 M.

Dissolve the ligand in PBS or a similar buffer, without other proteins or stabilizers. Avoid buffers with amino or sulfo-groups (e.g. Tris). High pH and high temperature during coating procedures are optimal conditions for quick formation of chemical bonds. (The upper pH and temperature limit is determined by the ligand). Buffers with slightly higher pH (e.g. borate buffer pH 9.5) may speed up the formation of covalent bonds between amino-groups in the ligand and the bead-surface. Such buffers can also be used to adjust the volume to give the wanted concentrations of beads, ligand and ammonium sulfate if necessary. Optimize the protocol for each ligand.

Note: Sugars or stabilizers may disturb the binding and should be removed from the ligand columns, prior to coupling.

It is recommended to incubate the ligand with the beads for 16–24 hours at 37°C with slow tilt rotation. Incubation with temperatures down to 4°C may be used for temperature sensitive ligands, but be aware that the covalent bond formation is slower and less efficient at low temperatures, and an additional 24 hours should be used to ensure covalent coupling.

Table 1: Recommended Buffers and Solutions

Buffer A: 0.1 M sodium phosphate buffer (pH 7.4)	2.62 g $NaH_2PO_4 \times H_2O$ (MW 137.99).14.42 g $Na_2HPO_4 \times 2H_2O$ (MW 177. 99). Dissolve in distilled water, adjust pH if necessary and adjust to 1 litre. This buffer is used for prewashing of the beads and should not be added any protein, sugar, etc.
Buffer B: 3 M ammonium sulfate (stock solution)	39.6 g $(NH_4)_2SO_4$ (MW 132.1). Dissolve in 0.1 M sodium phosphate buffer (pH 7.4) and adjust to 100 mL.
Buffer C: 0.1 M citrate pH 3.1	2.10 g citric acid ($C_6H_8O_7 \times H_2O$, MW 210.14). Dissolve in 90 mL distilled water, adjust to pH 3.1 and adjust to 100 mL.
Buffer D: 2 M NaI	3 g NaI (MW 149.9) to 10 mL distilled water.
Buffer E1: Phosphate buffered saline (PBS), pH 7.4	Cat. no. 10010-023.
Buffer E2: PBS with 0.1% BSA/HSA/ skimmed milk	Add 0.1% with BSA/HSA/skimmed milk (0.1g) to 100 mL Buffer E1.
Buffer E3: PBS/Tween® 20/Triton X	Add 0.5–1.0 % with Tween 20®/Triton X (50–100 mg) to 100 mL Buffer E1.

Elution of Isolated Target Protein

The target protein may be concentrated by elution in small volumes (down to 10 µL). Conventional elution methods can be applied for elution of target protein from the beads, e.g. low pH (2.8–3.5), change in ionic strength, affinity elution and boiling in SDS-PAGE buffer. The method of choice depends on affinity of the target protein to the protein ligand coupled onto the beads, target protein stability, downstream application and detection methods. To avoid eluting off non-specific binding to the tube walls, change tube before you elute. Most proteins will be eluted off at pH 3.1 but some protein functionality might be lost under such harsh conditions. If so, try milder elution conditions first (such as high salt, e.g. 2 M NaI, or step-wise elution reducing pH from 6 to 3). Use the same if the bead-bound ligand must remain functional to allow re-use of the beads. It is possible to re-use the ligand-coupled beads after mild elution by returning both beads and target protein to physiological pH (7.4) immediately after elution.

Protocols

Prepare the Beads

The freeze dried Dynabeads® M-270 Epoxy must be equilibrated in an appropriate buffer before coating. A sample of beads can be weighted out directly from the vial, using a micro-scale balance. Keep the vial in room temperature to avoid moisture that can deactivate the beads. Due to the short stability of hydrophilic epoxy groups in aqueous buffers we recommend to only prepare the wanted amount of beads for each experiment. The beads can also be resuspended in organic solvents like diglyme or DMF (see "Resuspend in Organic Solvent" section), in which the beads are stable for at least one year at 2°C to 8°C.

No. of beads	Weight (mg)
1 × 10^9	15
4 × 10^9	60 (content of 14301)
1 × 10^{10}	150
2 × 10^{10}	300 (content of 14302D)

Resuspend in Buffer A

This protocol is for resuspansion of ~3.3 × 10^8 beads (5 mg).

1. Carefully weigh out 5 mg beads (3.3 × 10^8) and resuspend in 1 mL Buffer A.
2. Vortex for 30 sec and incubate with tilting and rotation for 10 min.
3. Place the tube in a magnet for 1 min and discard the supernatant.
4. Remove the tube from the magnet and resuspend the washed beads in 1 mL Buffer A. Vortex for 30 sec.
5. Place the tube in a magnet for 1 min and discard the supernatant.
6. Go directly to "Couple Antibodies to the Beads".

Resuspend in Organic Solvent

Resuspension in an organic solvent like diglyme (diethylene glycol dimethyl ether) or DMF (dimethyl formamide) makes it easy to withdraw samples from the vial.

Note: Diglyme is toxic and flammable. Use a fume hood and gloves and take the necessary precautions when handling this solvent.

1. Add 2 mL diglyme to the vial of Cat. no. 14301 (4×10^9 beads) or 10 mL to the vial of Cat. no. 14302D (2×10^{10} beads) to give a final concentration of 2×10^9 beads/mL.
2. Before use, resuspend the beads well by vortexing for 1–2 min and transfer the wanted amount of beads to a tube with cap.
3. Place the tube on the magnet for 4 min. Pipet off the supernatant carefully, leaving beads undisturbed.
4. Go directly to "Couple Antibodies to Beads" section.

Couple Antibodies to Beads

- This protocol is for coupling of 5 mg (~3.3×10^8) beads or 165 µL (if the beads were resuspended in the organic solvent). It is generally not recommended to couple in lower volumes. For coupling of larger volumes, scale up the volumes accordingly, as shown in Table 2.
- Use ~100 µg ligand per 5 mg beads. Calculate the ligand volume from the concentration (µg ligand/mL). Example: For 5 mg beads, you need 100 µg ligand, so if the antibody conc. is 1 mg /mL you will need 100 µL ligand (100 µg :1000 µg/mL= 0.1 mL)
- The needed ammonium sulfate concentration is 1 M, but the stock solution in Buffer B is 3 M. To get the correct concentrations, add an equal volume of Buffer A, Buffer B and ligand, thus start by calculating the ligand volume.

1. Resuspend the dry beads in the same volume of Buffer A as calculated as the ligand volume (e.g. ~100 µL in our example). Mix or vortex.
2. Add 100 µg* ligand (~100 µL in our example), mix or vortex thoroughly before adding the same volume of Buffer B (100 µL in our example).
   Note: With 5 mg beads in a total of 300 µL, the coupling concentration is satisfactory with 1.1×10^9 beads/mL.
3. Incubate for 16–24 hours at 37°C with slow tilt rotation. Do not let the beads settle during the incubation period.
4. Place the tube on the magnet for 2 min. Gently turn the magnet upside-down twice, to ensure collection of any remaining beads in the cap. Remove the supernatant.
5. Wash the coated beads a total of four times with 1 mL Buffer E1 or E2*. Resuspend the beads and apply to a magnet for 2 min for each wash.
6. Resuspend the coated beads to the desired concentration in Buffer E1 or E2* (e.g 165 µL gives a bead concentration of ~2×10^9 beads/mL).
7. If the downstream application involves elution steps, physically adsorbed ligand can be removed by washing for 10 min in 0.5–1% with Tween® 20/Triton X-100 or similar non-ionic detergent.

* Blocking protein like BSA or skimmed milk powder should be added to 0.1–0.5% when this does not interfere with your downstream application.

Table 2: Volume overview for ligand coupling to Dynabeads® M-270 Epoxy.

Step	Reagent volumes	5 mg beads	20 mg beads
	Recommended tube	Microcentrifuge tube	Flow tube
	Recommended magnet	DynaMag™-2	DynaMag™-5
1	Add washed dry beads (or resuspended in organic solvent)	5 mg (165 µL)	20 mg (660 µL)
1	Resuspend the beads in Buffer A (1/3rd of total volume)	Same volume as calculated ligand	Same volume as calculated ligand
2**	Add ligand volume	Calculated from 100 µg	Calculated from 400 µg
2***	Add Buffer B	Same volume as calculated ligand	Same volume as calculated ligand
5	Wash 4 × in Buffer E1 or E2	~4 × 1 mL	~4 × 4 mL
6	Resuspend in Buffer E1 or E2 (optimize)	~1 mL	~4 mL

** Calculate the volume from the ligand (µg/mL) concentration. E.g if ligand is 1 mg/mL, add 100 µL ligand per 5 mg beads. *** Make sure the coupling concentration is within 1–2 × 10^9 beads/mL.

Isolate Target Molecule

Efficient isolation of target molecules is dependent on bead concentration, target molecule concentration, the ligand's affinity for the target molecule, and time. Equilibrium target-ligand binding is reached after approximately 1 hour. Binding is performed at 2°C to 8°C, at a recommended bead concentration of 1–10 × 10^9 beads/mL. This protocol is based on 5 mg beads, equivalent to 165 µL if the beads were resuspended in the organic solvent.

1. Add sample containing target molecule to 5 mg coated beads. For a 100 kDa protein use volume containing ~40 µg target molecule to assure an excess of this molecule.
2. Incubate with tilting and rotation for 1 hour to capture the target*.
3. Place the tube on the magnet for 4 min to collect the beads at the tube wall (for viscous samples, increase the time on the magnet). Pipet off the supernatant.
4. Wash beads 3 times using 1 mL Buffer E1 each time (see "Wash Beads").

* Incubation times as low as 10 min can be used with concentrated protein samples.

Target Elution Procedure

See "General Guidelines" for more information regarding elution.

1. Add an appropriate amount (e.g. 100 µL) of Buffer C to the beads with immobilized target.
2. Mix well by tilting and rotation for 2 min.
3. Place the test tube on the magnet and transfer the supernatant, containing purified target, to a clean tube.
4. To ensure re-use of the beads and functionality of the isolated target molecule, bring both beads and target back to physiological pH (7.4) immediately after elution.

Description of Materials

Dynabeads® M-270 Epoxy are uniform, superparamagnetic beads with a hydrophilic layer of glycidyl ether (epoxy) functional groups. These surface reactive epoxy groups allow for binding of proteins, peptides or other ligands, with covalent bond formation at neutral pH. Binding of ligands through amine or thiol groups occurs with no further activation of the surface.

Related Products

Product	Cat. no.
DynaMag™-2	12321D
DynaMag™-5	12303D
DynaMag™-15	12301D
HulaMixer® Sample Mixer	15920D
Dynabeads® Antibody Coupling Kit	14311D
Dynabeads® M-280 Tosylactivated	14203
Dynabeads® M-270 Carboxylic Acid	14305D
Dynabeads® M-270 Amine	14307D
Dynabeads® M-450 Tosylactivated	14013
Dynabeads® M-450 Epoxy	14011
Dynabeads® MyOne™ Tosylactivated	65501
Dynabeads® MyOne™ Carboxylic Acid	65011

REF on labels is the symbol for catalog number.

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