invitrogen™ | DYNAL®
invitrogen bead separations

Cat. no. 143.01
143.02D

Rev. no. 004

Dynabeads® M-270 Epoxy

For research use only

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1. PRODUCT DESCRIPTION

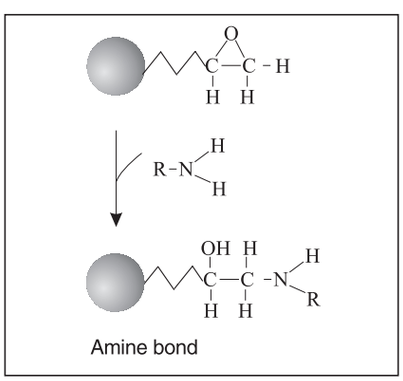
Dynabeads® M-270 Epoxy are uniform, superparamagnetic beads composed of highly cross-linked polystyrene with magnetic material precipitated in pores evenly distributed throughout the particles. The beads are further coated with a hydrophilic layer of glycidyl ether (epoxy) functional groups which seals the iron oxide inside the beads. 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.

2. PRINCIPLE

Dynabeads M-270 Epoxy act as a solid support in a wide variety of biomagnetic separations and manipulations. The hydrophilic surface ensures low non-specific binding, excellent dispersion abilities and easy handling of the beads in a wide variety of buffers.

Their size makes them particularly suitable for protein isolation for sample preparation, bioassays, selection of affinity binders etc. Due to the gentle pull of the beads to the magnet, they can also be used for selection of fragile cells. For cell separation in general, the larger Dynabeads (4.5 µm) are recommended.

Via primary amino or sulf-hydryl groups in the ligand, Dynabeads can be coated directly with proteins, peptides, antibodies, enzymes or other target specific molecules for the isolation of targets such as hormones, receptors, disease markers, bacteriophages etc. Alternatively, the bead-surface can be further modified to bind additional functional groups.



Amine bond

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, the beads are pulled to the side of the test tube by the use of a magnet (Dynal MPC™), allowing aspiration of unbound material. The magnet facilitates washing and target-concentration. Bead-bound targets can be used directly in bioassays, boiled in application buffer and analysed on SDS-PAGE. Alternatively, the target molecule can be eluted off the beads with conventional elution methods (high salt, low pH etc.).

3. INSTRUCTIONS FOR USE

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 microscale balance. 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, in which the beads are stable for at least one year at 2-8°C (see section 3.3. below).

3.1. Weight of Dynabeads M-270 Epoxy

Use the table below to be able to weigh out the wanted number of beads.

No. of beads	Weight (mg)
10^9	15
2 x 10^9	30
3 x 10^9	45
4 x 10^9	60 (content of 143.01)
5 x 10^9	75
10^{10}	150
1,5 x 10^{10}	225
2 x 10^{10}	300 (content of 143.02D)

3.2. Washing Procedure

A washing step is necessary to equilibrate the beads in appropriate buffer.

1. Add 0.1 M sodium phosphate buffer, pH 7.4 to the dry beads, to give a concentration of approximately 10^9 beads per ml.
2. Vortex for 30 seconds and incubate with mixing for 10 minutes.
3. Place the tube on a magnet (Dynal MPC) for 2 minutes and carefully pipette off the supernatant, leaving beads undisturbed.
4. Remove the test tube from the magnet and resuspend the beads carefully in the same volume with 0.1 M sodium phosphate buffer, pH 7.4 again. Vortex to mix properly.
5. Place the tube on the magnet for 2 minutes and pipette off the supernatant.
6. Resuspend the washed beads in the same buffer to a volume that will give the recommended bead concentration and ammonium sulfate concentration after addition of ligand solution and stock solution of ammonium sulfate (see below for recommended values). The beads are now washed and ready for coating.

3.3. Resuspension in Organic Solvent

Resuspension in an organic solvent like DMF (dimethyl formamide) makes it easy to withdraw samplesfrom the vial.

1. Add 2 ml diglyme to the 143.01-vial (contains 4 x 10^9 beads) or 10 ml to the 143.02-vial (contains 2 x 10^{10} beads) to give a final concentration of 2 x 10^9 beads/ml.
2. When samples are to be used: Resuspend the beads well in diglyme by vortexing for 1-2 minutes and transfer the wanted amount of beads to a tube with cap.
3. Place the tube on the magnet for 4 minutes. Pipette off the supernatant carefully, leaving beads undisturbed.
4. Equilibrate the beads (see procedure 3.2. above).

3.4. Coating Procedure

For coating with proteins or other ligands, it is recommended to 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. An overall final concentration of 1-2 x 10^9 beads per ml during incubation is recommended.

To enhance binding, a final concentration of ammonium sulfate of 1-3 M should be used during coating. The optimal ammonium sulfate concentration will depend 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 loose their function (some Igs) at molarities > 1.5 M.

The ligand should be dissolved in PBS or a similar buffer, without other proteins or stabilizers. Avoid buffers with amino or sulfo groups (e.g. Tris). 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.

Optimization of the protocol for each ligand is always recommended.

NOTE: Sugars or stabilizers may disturb the binding and should be removed from the ligand preparation by dialysis, spin columns, gel chromatography etc. prior to coupling.

Calculation example: 3 mg beads = 2 x 10^8 beads. The ligand requirement for 2 x 10^8 beads using 3 µg/10^7 beads (20 µg/mg beads) is 60 µg ligand. At a ligand concentration of 1 mg/ml, aliquote a volume of 60 µl. Wash and resuspend the beads in 60 µl 0.1 M phosphate buffer to adjust the bead-concentration and mix ligand and beads thoroughly. The needed ammonium sulfate concentration is 1 M. Add 60 µl (1/3 the final volume) of a 3 M stock-solution. The final volume is 180 µl, which gives a final bead concentration of 1.2 x 10^9 beads/ml (the ligand volume is then 1/3 of the final volume).

1. Make a homogeneous suspension of the washed beads as described in 3.2 above.
2. Add the calculated amount of ligand solution to the bead suspension. Vortex to ensure good mixing before adding the calculated ammonium sulfate stock solution.
3. Incubate 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. Do not let the beads settle during the incubation period.
4. Place the tube on the magnet for 4 minutes for magnetic separation. Carefully turn the magnet (with the tube in place) upside-down twice, to ensure collection of any beads that might remain in the cap. Remove the supernatant.
5. Wash the coated beads a total of four times with PBS or PBS with blocking protein. 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.
6. If the downstream application involves elution steps, physically adsorbed ligand can be removed by washing for 10 minutes in 0.5-1% (w/v) Tween 20/Triton X-100 or similar non-ionic detergent.
7. Resuspend the coated beads to the desired concentration in PBS or PBS with blocking protein. The beads are now ready for use.

3.5. Isolation of 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 2-8°C, at a recommended bead concentration of 1-10 x 10^9 beads/ml.

1. Add sample containing target molecule to the coated beads (2 x 10^8 beads). For 100 kD protein use volume containing approximately 25 µg target molecule to assure an excess of this molecule.
2. Incubate with tilting and rotation for one hour to capture the target (incubation times as low as 10 minutes can be used with concentrated protein samples).
3. Place the tube on the magnet for 4 minutes to collect the beads at the tube wall (for viscous samples, increase the time on the magnet). Pipette off the supernatant.
4. Wash beads 3 x using 1 ml PBS each time (see procedure 3.2. above).

3.6. Target Elution Procedure

Conventional elution methods can be applied for the elution of target protein from the beads. Low pH (2.8-3.5), change in ionic strength, affinity elution, electrophoresis, polarity reducing agents, deforming eluants can be applied, or even boiling the beads in SDS-PAGE application buffer for direct characterization of protein on SDS-PAGE. The method of choice depends on affinity of target molecule to the ligand coated onto the beads, stability of target molecule and downstream application and detection methods. Most proteins will be eluted off at pH 3.1 following the procedure described below, but some protein functionality might be lost under such harsh conditions. If maintaining functionality of the target molecule is essential, try milder elution conditions first (such as high salt , e.g. 2M NaI, or stepwise 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.

1. Add an appropriate amount (e.g. 60 µl) of 0.1 M citrate (pH 3.1) to beads with immobilized target.
2. Mix well by tilting and rotation for 2 minutes.
3. Place the test tube on the magnet and transfer the supernatant, containing purified target, to a clean tube.

To ensure reuse of the beads and functionality of the isolated target molecule, bring both beads and target back to physiological pH (7.4) immediately after elution.

4. GENERAL INFORMATION

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

4.1. Product Characteristics

Dynabeads M-270 Epoxy are sold as freeze dried beads. Each mg contains 6–7 x 10^7 beads. The product is available in two formats, 60 mg (Cat. no. 143.01) and 300 mg (Cat. no 143.02D).

Typical bead-characteristics for any given lot of this product:

Diameter:	2.8 µm
Specific surface area:	2-5 m^2/g
Active chemical functionality:	0.1 – 0.2 mmol/g

4.2. References:

1. Marfatia KA *et al.* Identification and characterization of the human MOG1 gene. Gene 2001, 266(1-2):45-56.
2. Hamm J, Alessi DR and Biondi RM. Bi-functional, substrate mimicking RNA inhibits MSK1-mediated cAMP-response element-binding protein phosphorylation and reveals magnesium ion-dependent conformational changes of the kinase. J.Biol.Chem. 2002, 277(48):45793-45802.
3. Laine S *et al.* In vitro and in vivo interactions between the hepatitis B virus protein P22 and the cellular protein gC1qR J. Virol. 2003 Dec; 77(23):12875-80.

4.3. Additional material needed

- Magnetic device (Dynal MPC™, Magnetic Particle Concentrator)
- Mixing/rotation device (Dynal® Sample Mixer or similar)
- Test tubes, glassware and pipettes
- Antibody/other selecting molecule
- Buffers/solutions (see below)

4.4. Recommended buffers/solutions

Invitrogen Dynal recommends the use of 0.1M Phosphate buffer pH 7.4, PBS and PBS Tween. Other buffers might be used, but buffers containing amino groups (e.g. Tris) should not be used for coating of ligand.

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). The molarity of salt in the final coating solution should never be less than 0.05M.

0.1M sodium phosphate buffer (pH 7.4):
2.62 g NaH_2PO_4 x H_2O (MW 137.99)
14.42 g Na_2HPO_4 x $2H_2O$ (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.

3M ammonium sulfate (stock solution):
39.6 g $(NH_4)_2SO_4$ (MW 132.1)
Dissolve in 0.1M sodium phosphate buffer (pH 7.4) and adjust to 100 ml.

0.1 M citrate pH 3.1:
2.10 g citric acid ($C_6H_8O_7$ x H_2O, MW 210.14)
Dissolve in 90 ml distilled water, adjust to pH 3.1 and adjust to 100 ml.

2 M NaI:
3 g NaI (MW 149.9) to 10 ml distilled water.

PBS pH 7.4 (phosphate buffered saline):
0.26 g NaH_2PO_4 x H_2O (MW 137.99)
1.44 g Na_2HPO_4 x $2H_2O$ (MW 177. 99)
8.78 g NaCl (MW 58.5)
Dissolve in 900 ml distilled water, adjust pH if necessary and adjust to 1 litre

PBS with 0.1% BSA/HSA/skimmed milk:
Include 0.1% (w/v) BSA/HSA/skimmed milk (0.1g) in 100 ml PBS (above).

PBS/Tween 20/Triton X:
Include 0.5-1.0 % (w/v) Tween 20/Triton X (50-100 mg) in 100 ml PBS (above).

4.5. Related Dynabeads Products

Cat. no.	Product name
142.03/04 & 301.01	Dynabeads® M-280 Tosylactivated
143.05D/06D	Dynabeads® M-270 Carboxylic Acid
143.07D/08D	Dynabeads® M-270 Amine
302.12	Dynabeads® M-450 Tosylactivated
302.02D/03D	Dynabeads® M-450 Epoxy
655.01/02/03	Dynabeads® MyOne™ Tosylactivated
650.11/12/13	Dynabeads® MyOne™ Carboxylic Acid

4.6. Storage & Stability

Precautions should be taken to prevent bacterial contamination of the beads.

When stored in unopened vials at 2-8°C, Dynabeads M-270 Epoxy are stable until the expiration date printed on the label.

The beads should be washed once before use. Beads should not be autoclaved, but can be incubated with ethanol (70%, 1 hour) or gamma irradiated.

Coated beads may be stored at 2-8°C for several weeks or even months, depending on the stability of the immobilized ligand. Coated beads should be washed once for 5 min in PBS/BSA before use. Use the magnet to collect the beads according to the washing procedure.

If a preservative is needed for storage of coated beads, a final concentration of 0.02% (w/v) sodium azide (NaN_3) may be added to the storage buffer. Carefully remove before use by washing (see 3.2 above). Required safety precautions must be followed when handling this cytotoxic material.

4.7. Warnings & Limitations

This product is for research use only. Not intended for any animal or human therapeutic or diagnostic use unless otherwise stated.

Sodium azide is 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.

Certificate of Analysis (CoA) is available upon request.

Material Safety Data Sheet (MSDS) is available at http://www.invitrogen.com.

4.8. Trademarks & Patents

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4.11. Warranty

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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