invitrogen

Dynabeads™ M-270 Epoxy

Catalog No. 14301, 14302D **Store at 2°C to 8°C**

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Product contents

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

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

Product description

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

The beads can be coated directly with proteins, peptides, antibodies, enzymes, or other target specific molecules via primary amino or sulfhydryl groups in the ligand.

Once coupled with ligand, the beads are added to a sample containing the target molecule. After a short incubation the target is captured by the beads. The beads are applied to a magnet, the unwanted supernatant is 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 eluted off the beads by conventional elution methods.

Note: The Dynabeads™ Antibody Coupling Kit is available as a complete antibody coupling solution, that includes Dynabeads™ M-270 Epoxy and buffers.

Required materials

- DynaMag™ Magnet (See thermofisher.com/magnets for recommendations)
- Sample mixer allowing tilting and rotation of tubes (e.g. HulaMixer™ Sample Mixer)
- Buffers and solutions (see Table 1)
- Antibody/other selecting molecule

General guidelines

- Use a mixer to tilt/rotate the tubes so Dynabeads™ magnetic beads do not settle at the tube bottom.
- Avoid air bubbles (foaming) during pipetting.
- Use the recommended volumes and incubation times.

Ligand coating guidelines

Optimize the coating protocol based on the characteristics of each type of ligand.

- Use >3 µg of pure ligand per 10^7 Dynabeads™ M-270 Epoxy for coupling of small ligands (such as peptides). Use slightly higher concentrations for coupling of larger ligands.
- Dissolve the ligand in PBS or a similar buffer, without other proteins or stabilizers.
- Avoid buffers with amino or sulfo-groups (e.g. Tris).
- Sugars or stabilizers can inhibit binding and should be removed from the ligand columns, prior to coupling.
- Use 1–2 × 10^9 beads/mL during incubation with the ligand.
- Use a final concentration of 1–3 M ammonium sulfate during coating. The optimal concentration depends on the nature of the ligand. Most proteins are sufficiently coated with 1 M ammonium sulfate, but hydrophilic or small ligands (peptide) require high concentrations (up to 3 M). Some biomolecules may lose functionality (i.e. some Igs) at molarities >1.5 M.
- 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 covalent bond formation becomes slower and less efficient. An additional 24 hours is necessary to ensure covalent coupling.
- High pH and high temperature during the coating process promotes formation of chemical bonds. (The upper limit of pH and temperature is determined by the ligand).
- Buffers with higher pH (e.g. borate buffer pH 9.5) can speed up formation of covalent bonds between amino-groups in the ligand and the bead-surface. Such buffers can also be used to adjust the volume and produce the desired concentrations of beads, ligand and ammonium sulfate if necessary.

Target elution guidelines

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, target protein stability, downstream application and detection methods.

Table 1 Recommended buffers and solutions[1]

Buffer A 0.1 M sodium phosphate buffer (pH 7.4) This buffer should not contain any added protein or sugar.	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.
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) Dissolve in 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.

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

- Most proteins can be eluted with pH 3.1 but some protein functionality may be lost under such harsh conditions. Mild elution conditions such as high salt (e.g. 2 M NaI), or step-wise elution by reducing pH from 6 to 3 may be preferable in such cases.
- Change tube before elution to avoid eluting off non-specific contaminants binding to the tube walls.
- It is possible to retain the functionality of the ligand, and re-use the ligand-coupled beads after mild elution by returning both beads and target protein to physiological pH (7.4) immediately after elution.
- The target protein may be concentrated by elution in small volumes (down to 10 µL).

Protocols

Prepare the beads

Equilibrate the freeze dried Dynabeads™ M-270 Epoxy beads in an appropriate buffer before coating. Weigh a sample of beads directly from the vial using a micro-scale balance. Keep the vial at room temperature and avoid moisture that can deactivate the beads. The stability of the hydrophilic epoxy groups is short for beads resuspended in aqueous buffers, and it is recommend to prepare only the required amount of beads for each experiment. Beads resuspended in organic solvents like diglyme or DMF (see "Resuspend beads in organic solvent"), are stable for at least one year if stored at 2°C to 8°C.

Resuspend beads in Buffer A

This protocol is for resuspansion of ~3.3 × 10^8 beads (5 mg). See “Description of materials” for more information on bead number and weight.

1. Weigh out 5 mg of lyophilized beads (3.3 × 10^8) and resuspend in 1 mL of Buffer A.
2. Vortex for 30 seconds and incubate with tilting and rotation for 10 minutes.
3. Place the tube in a magnet for 1 minute and discard the supernatant.
4. Remove the tube from the magnet and resuspend the washed beads in 1 mL of Buffer A, and vortex for 30 seconds.
5. Place the tube in a magnet for 1 minute and discard the supernatant.
6. Go directly to “Couple antibodies to the beads”.

Resuspend beads in organic solvent

Resuspension in an organic solvent like diglyme (diethylene glycol dimethyl ether) or DMF (dimethyl formamide) allow the beads to be easily removed 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 of 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 required amount of beads to a tube with cap.
3. Place the tube on a magnet for 4 minutes. Pipet off the supernatant carefully, leaving the beads undisturbed.
4. Go directly to “Couple antibodies to the beads”.

Couple antibodies to the beads

This protocol is for coupling of 5 mg (~3.3 × 10^8) of lyophilized beads or 165 µL of beads re-suspended in 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 of ligand per 5 mg of beads. Calculate the ligand volume based on the concentration (µg ligand/mL).

Example: For 5 mg of beads, you need 100 µg of ligand, so if an antibody concentration is 1 mg/mL you will need 100 µL ligand (100 µg:1000 µg/mL= 0.1 mL)

The final ammonium sulfate concentration for a typical coupling reaction is 1 M. To get the correct concentration, determine the desired ligand volume, and add equal volumes of Buffer A, Buffer B, and ligand.

1. Resuspend the lyophilized beads in the same volume of Buffer A as calculated for the ligand volume (e.g. ~100 µL in the example). Mix or vortex.
2. Add 100 µg ligand (~100 µL in the example), mix or vortex thoroughly before adding the same volume of Buffer B (100 µL in the example).
   Note: With 5 mg beads in a total of 300 µL, the coupling concentration is satisfactory at 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 minutes. Gently turn the magnet upside-down twice, to ensure collection of any beads adhering to 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 minutes 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, physically adsorbed ligand can be removed by washing for 10 minutes in 0.5–1% with Tween™ 20/Triton X-100 or similar non-ionic detergent.

* Add blocking protein like BSA or skimmed milk powder to a final concentration of 0.1–0.5% if it 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[1]	Add ligand volume	Calculated from 100 µg	Calculated from 400 µg
2[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

[1] Calculate the volume based on the ligand concentration (µg/mL). For example, if the ligand is 1 mg/mL, add 100 µL of ligand per 5 mg beads.

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

1. Add sample containing target molecule to 5 mg of coated beads. For a 100 kDa protein use a volume containing ~40 µg of target molecule to ensure an excess concentration.
2. Incubate with tilting and rotation for 1 hour to capture the target.
   Note: Incubation times as low as 10 minutes can be used with concentrated protein samples.
3. Place the tube on a magnet for 4 minutes 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.

Elute target

See "Target elution 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 minutes.
3. Place the test tube on a magnet and transfer the supernatant containing the 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.

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)

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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