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invitrogen bead separations

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Dynabeads® M-270 Amine

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1. PRODUCT DESCRIPTION

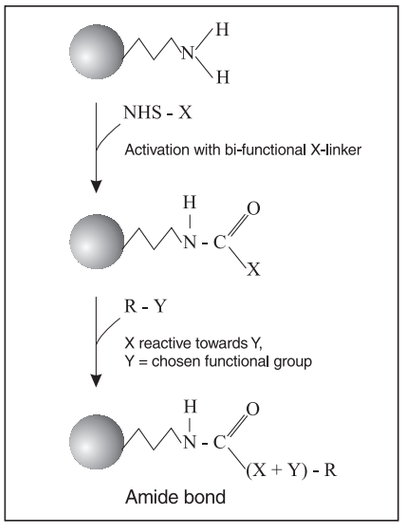
Dynabeads® M-270 Amine are uniform, superparamagnetic beads composed of highly cross-linked polystyrene with magnetic material precipitated in pores evenly distributed throughout the beads. The beads are further coated with a hydrophilic layer of glycidyl ether which seals the iron oxide inside the beads, and the surface is activated with primary amino functionality on a short hydrophilic linker.

The hydrophilic surface ensures low non-specific binding, excellent dispersion abilities and easy handling of the beads in a wide variety of buffers. The beads are sold in an aqueous suspension at a concentration of 2 x 10^9 beads/ml (approx. 30 mg/ml).

2. PRINCIPLE

Dynabeads M-270 Amine act as a solid support in a wide variety of biomagnetic separations and manipulations. 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.

Surface-reactive primary amino-groups allow immobilization of ligands such as proteins, peptides, carbohydrates or other target specific molecules through reductive amination of aldehyde or ketone groups without prior activation of the surface. Alternatively, ligands can be immobilized through amide-bond formation with carbodiimide-activated carboxylic acid groups. Bifunctional crosslinkers may be used to introduce other functional groups.



Dynabeads can be coated directly with peptides, carbohydrates, enzymes etc, for the isolation of targets such as hormones, receptors, lectines, disease markers, bacteriophages etc. 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

For ligand-coating of Dynabeads M-270 Amine, 3 µg pure ligand per 10^7 beads and a final concentration of 1-2 x 10^9 beads per ml is recommended.

Both ligand and bead concentration can be adjusted depending on the stock concentration, solubility and availability of your ligand. Higher bead concentrations increase the efficiency of immobilization, and higher ligand concentrations increase the loading on the beads.

Calculation example: 100 µl beads = 2 x 10^8 beads. Ligand requirement = 60 µg ligand (using 3 µg per 10^7 beads). A ligand concentration of 1-10 mg/ml gives a volume of 6-60 µl. The total volume should be 100-200 µl, and hence the beads should be dissolved in 40-200 µl before addition of the ligand.

3.A. - Washing procedure

Washing is necessary to equilibrate the Dynabeads M-270 Amine in an appropriate buffer.

1. Resuspend the beads well by pipetting and vortexing for 1-2 min. Avoid foaming.
2. Immediately pipette the volume to be used over to the desired test tube (e.g. 100 µl or 2 x 10^8 beads).
3. Place the tube on a magnet for 4 min. Carefully pipette off the supernatant, leaving beads undisturbed.
4. Remove the test tube from the magnet and carefully resuspend the beads in the original sample volume, using a buffer of choice according to the preferred conjugation method.
5. Repeat steps 3 and 4 once.
6. Resuspend the washed beads in the same buffer to a volume that will give the recommended bead concentration after addition of protein solution (see calculation example above). The beads are now ready for coating.

3.B. - Coating procedures

Alternative immobilization-principles:

- Direct ligand coupling via aldehyde- or ketone-groups by reductive amination (see section 3.B.1 below).
- Activation of the beads with a bi-functional crosslinker. An amine-reactive crosslinker will introduce a linker and a new functionality on the bead-surface for ligand coupling. The crosslinker should be chosen to give the preferred mode of immobilization of your specific ligand (see section 3.B.2 below).
- Activation of the ligand with carbodiimide or crosslinker. A crosslinker may also be introduced on the ligand. The crosslinker should be chosen so that the free end of the crosslinker is amine-reactive. Alternatively, carboxylic acid groups on the ligand may be activated with a carbodiimide and then reacted with the beads. This results in a direct amide bond formation between the beads and the ligand (see section 3.B.3 below).

3.B.1 - Coating procedure for conjugation of aldehyde or ketone containing molecules by reductive amination

Coupling of an aldehyde or ketone group in the ligand to amine on the bead surface can be achieved by Schiff base (imine) formation and reductive amination. Aldehyde groups can easily be prepared by sodium periodate oxidation of sugar residues in glycoproteins, or cleavage of carbon-carbon bonds with adjacent hydroxyl-groups in polysaccharides. The reductive amination is achieved by use of a reducing agent like cyanoborohydrid.

1. Dissolve the aldehyde containing ligand to a concentration of 1–10 mg/ml in an appropriate buffer (e.g. 0.1 M sodium phosphate buffer with 0.15 M NaCl, pH 7.4, or 0.1 M sodium borate pH 9.5 or 0.1 M sodium citrate pH 9.5). The same buffer should be used for pre-washing the beads.
2. Add the calculated amount of ligand solution to the homogenised bead-suspension. Vortex to ensure good mixing.
3. Add 1 µl of a solution containing 5 M cyanoborohydrid in 1 M NaOH (see section 4.3. below) to each 100 µl of reaction mixture, vortex properly and incubate for 2 hours at room temperature with slow tilt rotation. Do not let the beads settle during the incubation period. Note that cyanoborohydrid is highly toxic, use a fume hood and avoid contact with skin.
4. After incubation, place the tube on the magnet for 4 min and remove supernatant.
5. Add 0.1 M ethanolamine adjusted to pH 7.4 to the same volume as previously used and incubate for 15 min at room temperature with slow tilt rotation.
6. Wash the coated beads as described in section 3.C below.

3.B.2 - Activating with NHS-ester cross-linker

The most common kind of activating agents are NHS (N-hydroxy-succinimidyl)-esters. A large amount of different NHS-esters are commercially available for cross-linking. Depending on the nature of the cross-linker, this can react with chemical groups in the ligand to be immobilised. Reactivities include amine, sulfhydryl, carboxyl and hydroxyl as well as non-selective photoreaction.

NHS-ester cross-linkers must normally be prepared just prior to use. Use a 10-fold molar excess compared to the amount of ligand to be immobilised.

1. Resuspend the beads in 0.1 M sodium phosphate buffer with 0.15 M NaCl, pH 7.4. Avoid amine containing buffers like Tris or glycine as they will compete with the NHS-ester reaction.
2. Dissolve the NHS-ester according to the manufacturer's instruction and add the required volume to the bead-solution. Water-soluble NHS-esters may be added directly to the beads. Final volume should be equal to the bead-volume originally pipetted from the vial. Vortex to mix properly.
3. Incubate for 30 min at room temperature with slow tilt rotation.
4. After incubation, place the tube on the magnet for 4 min and remove the supernatant. Wash twice with the buffer above.
5. The beads are now activated and with a functional group according to the chosen cross-linker.

3.B.2.1 - Coating after activation with NHS-ester cross-linker with amine-reactivity:

With homo-bifunctional cross-linkers a second NHS-ester group will react similarly with primary amines in the ligand, and hence these are normally used for immobilisation of proteins or the N-terminal in peptides.

After activating the beads as described in section 3.B.2 above:

1. Make a homogeneous suspension of the activated beads. If necessary, use the same buffer to adjust the volume to the required bead-concentration.
2. Add the calculated amount of ligand. Vortex to ensure good mixing.
3. Incubate for 30 min at room temperature or 2 hours at 4°C with slow tilt rotation.
4. After incubation, place the tube on the magnet for 4 min and remove the supernatant.
5. Add 0.05 M Tris pH 7 and incubate for 15 minutes at room temperature with slow tilt rotation, to quench non-reacted groups.
6. Wash the coated beads as described in section 3.C below.

3.B.2.2 - Coating after activation with NHS-ester cross-linkers with sulfhydryl-reactivity:

Sulfhydryl reactive groups are often pyridyldithio or iodo/bromoacetyl. For oligonucleotides maleimide may also be used. Appropriate buffers and incubation conditions should be used to give reaction with sulfhydryl. The ligand must contain free sulfhydryl groups in order to be immobilised.

After activating the beads as described in section 3.B.2 above:

1. Make a homogeneous suspension of the activated beads. Use a buffer according to the active group chosen (see table below). If necessary, use the same buffer to adjust the volume to the required bead/ligand concentration.
2. Add the calculated amount of free sulfhydryl-containing ligand. Vortex to ensure good mixing.
3. Incubate for the recommended time and temperature with slow tilt rotation.
4. After incubation, cysteine may be added to a final concentration of 5 mM to quench non-reacted groups. Incubate for 15 min at room temperature with slow tilt rotation.
5. Wash the coated beads as described in section 3.C below.

3.B.2.3 - Coating after activation with NHS-ester cross-linkers with photoreactivity:

SH-reactive group	Recommended buffer	Incubation conditions
Maleimide	0.1 M sodium phosphate pH 6.5-7.5	2 hours room temperature or 4 hours at 4°C
Iodo/Bromo-acetyl	0.05 M sodium borate pH 8.3	1 hour at room temperature Protect from light
Pyridyldithio	Phosphate buffered saline (PBS) pH 7.5	Over night at room temperature

Photoreactive groups like hydroxylphenylazide, nitrophenylazide, phenylazide or perfluoroarylazide moiety may be used to immobilise ligands with amine groups. Activation with a cross-linker with photoreactive groups must be performed under dark-room conditions!

After activating the beads as described in section 3.B.2 above:

1. Make a homogeneous suspension of the activated beads. If necessary, use the same buffer to adjust the volume to the required bead/ligand concentration.
2. Add the calculated amount of ligand. Vortex to ensure good mixing.
3. Irradiate with light with the appropriate wavelength using the recommended time and temperature conditions with slow tilt rotation. Note that the beads may quench the light, and hence the method may have to be optimised.
4. Wash the coated beads as described in section 3.C below.

NOTE: The beads are compatible with organic solvents such as DMF, and both the activation and coupling steps described above can be performed in dry solvents. This will eliminate the competing hydrolysis reaction, thus higher yields may be achieved by using longer reaction times. Wash once in cold, purified water before transferring the beads form organic solvent to a salt-containing buffer.

3.B.3 - Activation with cross-linkers with amine- and carboxyl- reactivity, for coating with carboxyl-containing ligand

Amine-groups on the surface of the beads and carboxyl-groups in the ligand may be linked together by use of EDC or EDC/NHS (or other carbodiimides) under the condition where no other primary amino-groups are present in the ligand. EDC reacts with carboxyl-groups to form an amine-reactive intermediate. This intermediate is unstable in aqueous solutions. To stabilise, NHS may be introduced.

1. Wash the beads with 0.1 M MES (2-[N-morpholino]ethane sulfonic acid), pH 4.5-5 (or 0.1 M MES, 0.5 M NaCl pH 6.0).
2. Dissolve the ligand in the same buffer to a concentration of 1-10 mg/ml and add the recommended amount of ligand. Vortex to ensure good mixing.
3. Dissolve 10 mg EDC in 1 ml cold deionized water, (or dissolve 10 mg EDC and 15 mg NHS per ml). This must be done immediately prior to use.
4. Add 50-100 µl EDC (or EDC/NHS) solution for each mg ligand used. Vortex to mix properly.
5. Incubate for 2 hours at room temperature or 2 hours at 4°C with slow tilt rotation.
6. Add hydroxylamine (NH2OH x HCl, MW 69.49) to a final concentration of 10 mM to quench the reaction, and incubate for 15 minutes at room temperature with slow tilt rotation.
7. Wash the coated beads as described in protocol 3.C below.

3.C. - Washing of coated beads

All immobilization procedures require washing of the coated beads to remove excess ligand.

1. Wash the coated beads a total of four times in PBS or similar. It is recommended to add BSA to 0.5% in the washing buffer for effective blocking of the surface (when this does not interfere with the downstream applications). Use 0.1% Tween-20 or Triton X-100 as an alternative or in addition to the BSA.
2. Resuspend the coated beads in PBS with 0.1% BSA and / or 0.01% Tween-20 / Triton X-100 to 1 x 10^9 beads / ml.
3. For storage of the coated beads, add sodium azide to 0.02% and store at 2-8°C. Other bacteriostatic agents can be used. Coated beads can usually be stored for several months, depending on the stability of the immobiilized ligand. If the coated beads are stored for more than two weeks, wash twice for five minutes with a buffer suitable for your application prior to use.

3.D. - Isolation of target molecule

Efficient isolation of target molecules is dependent on the concentration of beads and target molecules, the ligand's specific affinity and time.

1. Remove the storage buffer from 200 µl beads (2 x 10^8 beads). Add 100-200 µl of the solution containing your target molecule. Excess target is needed if maximum binding is required (Eg. for a 100 kD protein, use 25 µg.)
2. Incubate with tilting/rotation for up to 1 hour to capture the target. Higher target and/or bead concentration increases the rate of binding.
3. Place the tube on the magnet for 4 min. to collect the beads at the tube wall (for viscous samples, increase the separation time). Pipette off the supernatant.
4. Wash the beads 3 times (using 1 ml PBS each time) by the use of the magnet.

3.E. - Target protein elution procedure

Conventional elution methods can be applied. 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 protein characterization. The method of choice will depend on the affinity of the target molecule to the ligand coated onto the beads, stability of the target molecule as well as downstream applications and detection methods. Most proteins will be eluted off the beads 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 important, try milder elution conditions first, e.g. high salt (e.g. 2M NaI) or a stepwise elution reducing pH from 6 to 3. This approach is also recommended if the bead-bound ligand must remain functional to allow reuse of the beads.

1. Add 30 µl 0.1 M citrate (pH 3.1) to beads with immobilised target. Mix well by tilting and rotation for 2 minutes.
2. Place the test tube on a magnet and transfer the supernatant, containing purified target, to a clean tube.
3. Add 30 µl 0.1 M citrate (pH 3.1) to the beads to elute any remaining target. Mix well by tilting and rotation for 2 minutes.
4. Place the test tube on a magnet, pipette off the eluate and pool the supernatants containing pure target molecules. Total collected volume = 60 µl.

To ensure reuse of the beads and functionality of the isolated target molecules, 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

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

Diameter:	2.8 µm
Specific surface area:	2-5 m2/g
Active chemical functionality:	0.1 – 0.2 mmol/g
Surface charge:	Positive at pH 2-9

4.2. Additional material needed

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

4.3. Recommended buffers/solutions

Invitrogen Dynal recommends the use of 0.1M Phosphate buffer pH 7.4, PBS and PBS Tween. Other buffers can also 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 with 0.15 M NaCl:
2.62 g NaH_2PO_4 x H_2O (MW 137.99)
14.42 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.

0.1 M Sodium borate pH 9.5:
0.62 g H_3BO_3 (MW 61.83)
Dissolve in 90 ml distilled water, adjust to pH 9.5 and adjust to 100 ml.

0.05 M Sodium borate pH 8.3:
0.30 g H_3BO_3 (MW 60.83)
Dissolve in 90 ml distilled water, adjust to pH 8.3 and adjust to 100 ml.

0.1 M Sodium citrate pH 9.5.
2.94 g $C_6H5O_7Na_3$ x $2H_20$ (MW 294)
Dissolve in 90 ml distilled water, adjust to pH 9.5 and adjust to 100 ml.

5 M Cyanoborohydrid in 1 M NaOH (highly toxic, avoid contact):
0.31 g $NaCNBH_3$ (MW 62.84)
Dissolve in 10 ml 1 M NaOH (40.0 g NaOH (MW 40.0) to 1 litre distilled water).

0.1 M Ethanolamine adjusted to pH 7.4:
0.6 ml C_2H_7NO (MW 61.08, d = 1.02 g/ml)
Dissolve in 90 ml distilled water, adjust to pH 7.4 and adjust to 100 ml.

0.05 M Tris pH 7.4:
0. 79 gTris HCl (MW 157.6)
Dissolve in 90 ml distilled water, adjust to pH 7.4 and adjust to 100 ml.

0.1 M MES pH 4.5-5:
2.13 g MES (MW 213.25)
Dissolve in 90 ml distilled water, adjust to pH 4.5 - 5 and adjust to 100 ml.

0.1 M MES, 0.5 M NaCl pH 6:
2.13 g MES (MW 213.25)
2.93 g NaCl (MW 58.5)
Dissolve in 90 ml distilled water, adjust to pH 6.0 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:
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% (w/v) 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).

4.4. Storage & Stability

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

When stored in unopened vials at 4°C, Dynabeads M-270 Amine are stable until the expiration date printed on the label.

The beads should be washed once before use (see section C above). Beads should not be autoclaved, but can be incubated with ethanol (70%, 1 hour) or gamma irradiated after freeze drying.

Antibody-coated beads may be stored at 4°C for several weeks without loss of antigen binding capacity. Coated beads should be washed once for 5 min in PBS/BSA before use.

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. Required safety precautions must be followed when handling this cytotoxic material.

4.5. Warnings & Limitations

For research use only. Not for use in human diagnostic or therapeutic procedures.

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.

Cyanoborohydrid is highly toxic. Use a fume hood and avoid contact with skin.

If cytotoxic preservatives are added these must be carefully removed before use by washing.

Certificate of Analysis (CoA) is available upon request. Material Safety Data Sheet (MSDS) is available at 'www.invitrogen.com'.

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4.8. 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. This warranty shall not apply to any products which shall have been altered outside Invitrogen Dynal, nor shall it apply to any products which have been subjected to misuse or mishandling. ALL OTHER WARRANTIES, EXPRESSED, IMPLIED OR STATUTORY, ARE HEREBY SPECIFICALLY EXCLUDED, INCLUDING BUT NOT LIMITED TO WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. Invitrogen Dynal's maximum liability is limited in all events to the price of the products sold by Invitrogen Dynal. IN NO EVENT SHALL INVITROGEN DYNAL BE LIABLE FOR ANY SPECIAL, INCIDENTAL OR CONSEQUENTIAL DAMAGES. Some states do not allow limits on warranties, or on remedies for breach in certain transactions. In such states, the limits set forth above may not apply.

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