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Dynabeads® M-270 Amine

Catalog nos. 14307D, 14308D **Store at 2˚C to 8˚C**

Rev. Date: May 2012 (Rev. 004)

Product Contents

Cat. no.	Volume
14307D	2 mL
14308D	10 mL

Dynabeads® M-270 Amine contains ~2 × 10^9 beads (30 mg)/mL in an aqueous suspension.

Product Description

Dynabeads® M-270 Amine acts as a solid support in a wide variety of biomagnetic separations. Their size makes them particularly suitable for protein isolation for sample preparation, bioassays, selection of affinity binders etc. Surface-reactive primary amino-groups allow immobilization of ligands 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. Bi-functional cross-linkers may be used to introduce other functional groups.

Dynabeads® M-270 Amine can be coated directly with peptides, carbohydrates, enzymes etc, for the isolation of different targets, e.g. hormones, receptors, lectins, disease markers, 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 target bound beads are washed to give a pure sample. Bead-bound targets can be used directly in bioassays, and analyzed on SDS-PAGE. Alternatively, the target molecule can be eluted off the beads with conventional elution methods.

Required Materials

- Magnet (DynaMag™ portfolio). See www.lifetechnologies.com/magnets for recommendations.
- Mixer allowing tilting and rotation of tubes (e.g. HulaMixer® Sample Mixer).
- Antibody/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.
- 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 0.05 M.

Protocols

For ligand-coating of Dynabeads® M-270 Amine, we recommend using 3 µg pure ligand per 1 × 10^7 beads and a final concentration of 1–2 × 10^9 beads per mL. Both ligand and bead concentration can be adjusted according to your needs. Higher bead concentrations increase the efficiency of immobilization, and higher ligand concentrations increase the loading on the beads.

Calculation example: 100 µL beads = 2 × 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.

Wash the Dynabeads®

This protocol is used prior to the coating procedure. See above for recommended volumes.

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 Buffer*, or at least 1 mL, and resuspend.
4. Place the tube in a magnet for 1 min and discard the supernatant.
5. Repeat steps 3–4 once.
6. Remove the tube from the magnet and resuspend the washed beads in the preferred Buffer*. Use a volume that gives the optimal bead concentration after the addition of protein solution (see example above).

** The Buffer required depends on the preferred conjugation procedure.*

Coating Procedures

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 cyanoborohydride.

Table 1: Recommended Buffers and Solutions

Coating Buffer: 0.1 M sodium borate 0.1 M sodium phosphate buffer pH 7.4 with 0.15 M NaCl	2.62 g $NaH_2PO_4 \times H_2O$ (MW 137.99); 14.42 g $Na_2HPO_4 \times 2H_2O$ (MW 177. 99), 8.78 g NaCl (MW 58.5). Dissolve in 900 mL distilled water, adjust pH if necessary and adjust the volume 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 the volume 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 the volume to 100 mL.
0.1 M sodium citrate pH 9.5.	2.94 g $C_6H_5O_7Na_3$ x $2H_20$ (MW 294). Dissolve in 90 mL distilled water, adjust to pH 9.5 and adjust to 100 mL.
5 M cyanoborohydride in 1 M NaOH (highly toxic, avoid contact)	Dissolve 0.31 g $NaCNBH_3$ (MW 62.84) 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 the volume 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 the volume 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 the volume 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 the volume to 100 mL.
0.1 M citrate pH 3.1	2.10 g citric acid (C_6H8O_7 x H_2O, MW 210.14). Dissolve in 90 mL distilled water, adjust to pH 3.1 and adjust the volume to 100 mL.
2 M NaI	Add 3 g NaI (MW 149.9) to 10 mL distilled water.
PBS pH 7.4	0.26 g $NaH_2PO_4 \times H_2O$ (MW 137.99). 1.44 g $Na_2HPO_4 \times 2H_2O$ (MW 177. 99). 8.78 g NaCl (MW 58.5). Dissolve in 900 mL distilled water, adjust pH if necessary and adjust the volume to 1 litre.
PBS with 0.1% with BSA/HSA/ skimmed milk	Include 0.1% with BSA/HSA/skimmed milk (0.1g) in 100 mL PBS (above).
PBS/Tween®-20/Triton X-100	Include 0.5–1.0% with Tween® 20/Triton X (50–100 mg) in 100 mL PBS (above).

1. Dissolve the ligand to a concentration of 1–10 mg/mL in an appropriate buffer*.
2. Add the calculated amount of ligand to the resuspended beads and vortex.
3. Add 1 µL of a solution containing 5 M cyanoborohydride in 1 M NaOH** per 100 µL of reaction mixture, vortex properly and incubate for 2 hours at room temperature with slow tilt rotation.
4. 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 and rotation.
6. Wash the coated beads as described in "Wash the Dynabeads®", just replacing the buffers with PBS with 0.5% BSA, 0.01% Tween®-20, and 0.02% sodium azide (optional – as preservative) and resuspend to a bead concentration of 1 × 10^9 beads/mL.

Caution: Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

- 0.1 M sodium phosphate buffer w/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.
- Cyanoborohydride is highly toxic, use a fume hood and avoid contact with skin.

Activate with NHS-ester cross-linker

The most common kind of activating agents are NHS (N-hydroxy-succinimidyl)-esters. Depending on the nature of the cross-linker, this can react with chemical groups in the ligand to be immobilized. Reactivities include amine, sulfhydryl, carboxyl, and hydroxyl as well as non-selective photoreaction. NHS-ester cross-linkers must normally be prepared immediately before 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 and vortex. Water-soluble NHS-esters maybe added directly to the beads. Final volume should be equal to the bead-volume originally pipette from the vial.
3. Incubate for 30 min at room temperature with slow tilt and rotation.
4. 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.

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 immobilization of proteins or the N-terminal in peptides. After activating the beads as described in the section:

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 min at room temperature with slow tilt rotation, to quench non-reacted groups.
6. Wash the coated beads as described in step 6 of section:"Coating procedure for conjugation of aldehyde or ketone containing molecules by reductive amination".

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 to be immobilised.

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 and vortex.
3. Incubate for the recommended time and temperature with slow tilt and 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 and rotation.
5. Wash the coated beads as described in step 6 of section: "Coating procedure for conjugation of aldehyde or ketone containing molecules by reductive amination".

Coating after activation with NHSester cross-linkers with photoreactivity.

SH-reactive group	Recommended buffer	Recommended buffer
Maleimide	0.1 M sodium phosphate pH 6.5–7.5	2 hours room temperature or 4 hours at 4°C.
Iodo/Bromoacetyl	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 perfluoro-arylazide 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 "Activating with NHS-ester cross-linker":

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 and vortex.
3. Irradiate with light with the appropriate wavelength using the recommended time and temperature conditions with slow tilt rotation.
   Note: The beads may quench the light, and hence the method may have to be optimized.
4. Wash the coated beads as described in step 6 of section: "Coating procedure for conjugation of aldehyde or ketone containing molecules by reductive amination".

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.

Activation with cross-linkers with am}ine- 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 stabilize, 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 and vortex.
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 and vortex.
5. Incubate for 2 hours at room temperature or 2 hours at 4°C with slow tilt rotation.
6. Add hydroxylamine ($NH_2OH \times HCl$, MW 69.49) to a final concentration of 10 mM to quench the reaction, and incubate for 15 min at room temperature with slow tilt rotation.
7. Wash the coated beads as described in step 6 of section:"Coating procedure for conjugation of aldehyde or ketone containing molecules by reductive amination".

Isolate 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 (2×10^8 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 kDa 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. Pipet off the supernatant.
4. Wash the beads 3 times (using 1 mL PBS each time) by the use of the magnet.

Target Protein Elution

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. Most proteins will be eluted off the beads at pH 3.1, 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. 2 M 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 immobilized target. Mix well by tilting and rotation for 2 min.
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 min.
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.

Description of Materials

Dynabeads® M-270 Amine are uniform, superparamagnetic beads (2,7 µm in diameter) coated with a hydrophilic layer of glycidyl ether the surface is activated with primary amine 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.

Related Products

Product	Cat. no.
DynaMag™-2	12321D
DynaMag™-5	12303D
HulaMixer® Sample Mixer	15920D
Dynabeads® M-280 Tosylactivated	14203
Dynabeads® M-270 Carboxylic Acid	14305D
Dynabeads® MyOne™ Tosylactivated	65501
Dynabeads® MyOne™ Carboxylic Acid	65011

REF on labels is the symbol for catalog number.

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