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Dynabeads® M-270 Carboxylic Acid

Catalog nos. 14305D, 14306D **Store at 2˚C to 8˚C**

Rev. Date: June 2012 (Rev. 005)

Product Contents

Cat. no.	Volume
14305D	2 mL
14306D	10 mL

Dynabeads® M-270 Carboxylic Acid contains ~2 × 10^9 beads (approx. 30 mg)/mL supplied in purified water.

Product Description

Dynabeads® M-270 Carboxylic Acid is designed to act as a solid support for a wide variety of biomagnetic separations. Their size makes them particularly suitable for isolation of proteins or fragile cells. The very rapid and gentle coupling chemistry of the ligand-immobilization reaction make them very useful in coupling labile proteins, peptides, and functional enzymes for the isolation of a wide variety of targets (e.g. hormones, receptors, disease markers, etc). For cell separation in general, we recommend using the larger 4.5 µm Dynabeads®.

Activation of the Dynabeads® M-270 Carboxylic Acid can be performed with a carbodiimide followed by coupling of an amine containing ligand. Alternatively, a bifunctional cross-linker may be used to introduce other functional groups like thiol, amine, maleimide, etc. If the ligand to be bound is an oligonucleotide, it does not contain a primary amino function. This can be introduced by e.g. using 5'-amino modified oligonucleotides.

Note that other amino-groups in the oligonucleotide might to some degree react with the carboxylic acid groups on the beads, resulting in coupling via the internal bases. Once coupled with your ligand, the Dynabeads® can be added to a cell lysate or other suspensions containing your target molecule. After a short incubation, the Dynabeads® are quickly pulled to the side of the test-tube by the use of a magnet. Dynabeads® with bound target molecule can be used directly in downstream bioassays, or can be boiled in application buffer and analyzed on SDS-PAGE. Alternatively, the target molecule can be eluted off the Dynabeads® 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).
- Ligands.
- Buffer/solutions (see Table 1).

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.

Decide Activation Protocol

The traditional procedure for ligand coupling is the formation of an amide bond between a primary amino group of the ligand and the carboxylic acid groups on the surface of the Dynabeads®, mediated by carbodiimide activation. The intermediate product of the reaction between the carboxylic acid and the carbodiimide is very labile and will hydrolyse quickly. To get the desired immobilization of the ligand it is therefore important to have the ligand immediately available. Alternatively, the activated Dynabeads® can be captured as a less labile intermediate, like an N-hydroxyl succinimide ester, and then react with the ligand over a longer period. There are several alternative protocols for carbodiimide-mediated immobilization of ligand by amide bond formation:

There is a one-step protocol, a two-step protocol (without NHS), and a two-step protocol with NHS. The one-step protocol is recommended when using ligands not containing carboxylic acid groups (e.g. oligonucleotides). If the ligand contains carboxylic acid groups, these may react with the carbodiimide and cause polymerization of the ligand. Since this method is less laborious and generally gives higher yields, it may however still be advantageous to use this method if it is possible to add ligand in excess to compensate for the loss due to polymerization.

The two-step protocol is preferred when the ligand contains carboxylic acid groups and you have limited amounts of the ligand available. The two-step protocol without NHS requires a very fast wash of the beads in cold buffer after the activation. The two-step protocol with NHS should be used if the ligand is in an alkaline buffer or a buffer with high phosphate concentration. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC, MW 191.7) is the carbodiimide most commonly used. It is very soluble in water and relatively stable as an aqueous solution. For some applications, other carbodiimides may slightly improve the results.

Table 1: Recommended Buffers and Solutions

0.01 M NaOH	0.4 g NaOH (MW 40.0) dissolved in 1000 mL distilled water.
100 mM MES, pH 5	2.13 g MES (2-[N-morpholino]ethane sulfonic acid, MW 213.25). Dissolve in 90 mL distilled water, adjust to pH 5 and adjust to 100 mL.
0.05 M Tris, pH 7.4	0. 79 g Tris HCl (MW 157.6). Dissolve in 90 mL distilled water, adjust to pH 7.4 and adjust to 100 mL.
0.1 M Citrate, pH 3.1	2.10 g citric acid (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)	Cat. no. 10010-023.
PBS with 0.1% (w/v) BSA/HSA/ skimmed milk	Include 0.1% with BSA/HSA/skimmed milk (0.1 g) in 100 mL PBS.
PBS/Tween® 20/Triton X-100	Include 0.5–1.0 % with Tween® 20/Triton X (50–100 mg) in 100 mL PBS. Add a final concentration of 0.02% with sodium azide to the Dynabeads® storage buffer, if preferred. **Caution:** Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

Protocols

For coating of ligand to Dynabeads® M-270 Carboxylic Acid, use ~20 µg pure protein or ~700 pmol oligonucleotides/peptides per mg Dynabeads®, and a final concentration of 10-30 mg beads/mL during incubation.

The protocols described the use of 3 mg Dynabeads®, but can be directly scaled up or down. The protocols are generic and should be optimized for your application (e.g. sample volume, concentration of ligand/ beads/EDC, MES buffer volume, and pH).

Calculations per 3 mg beads:

- Bead volume (30 mg beads/mL): 3 mg beads = 100 µL.
- Protein volume (20 µg protein/mg beads) = 60 µg = 60 µL (when protein conc. is 1 mg/mL).
- The required amount of EDC varies depending on the performed coating procedures.
- The final sample volume should be 100 µL to obtain the recommendation Dynabeads® concentration of 30 mg/mL.

Activate and Couple Ligands to Dynabeads® M-270 Carboxylic Acid

Read "Decide Activation Protocol" to chose the correct coating procedure.

One-Step Coating Procedure

1. Thoroughly resuspend the Dynabeads® in the vial and transfer 100 µL to a new tube.
2. Wash the beads by adding 100 µL 25 mM MES, pH 5 and incubate for 10 min with good mixing.
3. Apply the beads to the magnet for 2 min and remove the supernatant.
4. Repeat steps 2–3 once.
5. Add 60 µg ligand (60 µL in our example) in 60 µL 25 mM MES, pH 5 to the washed Dynabeads®. Mix well and incubate with slow tilt rotation at room temperature for 30 min.
6. Immediately before use dissolve EDC in cold 100 mM MES, pH 5 to a concentration of 100 mg/mL.
7. Add 30 µL EDC solution (3 mg) to the Dynabeads®/ligand suspension. Mix well.
8. Add 10 µL of 25 mM MES, pH 5 to final volume of 100 µL.
9. Incubate for 2 hours or longer at 4°C with slow tilt rotation.
10. Wash the coated Dynabeads® as described in "Wash Dynabeads®".

Two-Step Coating Procedure (without NHS)

Activation with EDC

1. Thoroughly resuspend the Dynabeads® in the vial and transfer 100 µL to a new tube.
2. Wash the beads by adding 100 µL 0.01 M NaOH and incubate for 10 min with good mixing.
3. Apply the beads to the magnet for 2 min and remove the supernatant.
4. Repeat steps 2–3 once.
5. Repeat steps 2–3 three more times, replacing 0.01 M NaOH with 100 µL deionized water.
6. Apply to the magnet for 2 min and remove the supernatant.
7. Dissolve the EDC in cold, deionized water to 19–76 mg/mL (0.1–0.4 M). Add 100–200 µL of EDC-solution to the Dynabeads® and vortex.
8. Incubate for 30 min at room temperature with slow tilt and rotation.

9. After incubation, place the tube on the magnet for 2 min and remove the supernatant.
10. Wash once with cold, deionized water and once with 50 mM MES, pH 5, as quickly as possible to avoid hydrolysis of the activated carboxylic acid groups.

The Dynabeads® are now activated and ready for coating with a ligand containing primary amine groups. Activated beads cannot be stored and you should proceed directly to the next step.

Immobilization of ligand after activation

1. Apply to a magnet for 2 min and remove the supernatant.
2. Add 60 µg ligand (60 µL in our example) in 60 µL 50 mM MES, pH 5 to the activated Dynabeads®.
3. Add 40 µL of 50 mM MES, pH 5 to final volume of 100 µL and vortex.
4. Incubate for at least 30 min at room temperature, or 2 hours at 4°C, with slow tilt and rotation.
5. After incubation, place the tube on the magnet for 2 min and remove the supernatant.
6. Wash the coated Dynabeads® as described in "Wash Dynabeads®".

Two-Step Coating Procedure using NHS

Activation with EDC and NHS

1. Wash Dynabeads® M-270 Carboxylic Acid twice with 25 mM MES, pH 5, using the equal volume of Dynabeads® (100 µL) pipetted out of the vial, for 10 min with good mixing.
2. Immediately before use dissolve EDC in cold 25 mM MES, pH 5 to a concentration of 50 mg/mL.
3. Similarly, prepare a 50 mg/mL solution of NHS in 25 mM MES, pH 5.
4. Add 50 µL of EDC solution and 50 µL of NHS solution to the washed Dynabeads®. Mix well and incubate with slow tilt rotation at room temperature for 30 min.
5. After incubation, place the tube on the magnet for 4 min and remove the supernatant. Wash twice with 100 µL of 25 mM MES, pH 5.

The Dynabeads® are now activated and ready for coating with a ligand containing primary amine groups.

Immobilization of ligand after activation

1. Add the required amount of ligand (60 µg) in 25 mM MES, pH 5 (60 µL) to activated Dynabeads®.
2. Add 40 µL 25 mM MES, pH 5 to final volume of 100 µL. Vortex to ensure good mixing.
3. Incubate for at least 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. Wash the coated Dynabeads® as described above (see "Wash Dynabeads®").

Isolate Target Molecule

Efficient isolation of target molecules using Dynabeads® is dependent on the bead concentration, target-molecule concentration, the ligand's affinity for the target molecule, and time. Binding is performed from 10–60 min, at a recommended concentration of 1–10 × 10^9 beads/mL. Target-ligand equilibrium is reached after approximately 1 hour.

1. Add the sample containing ~25 µg target molecule (for a 100 kDa protein) to the coated Dynabeads® (3 mg beads).
2. Incubate for one hour at room temperature with tilting and rotation*.
3. Place the tube on the magnet for 2 min and remove the supernatant.
4. Wash the Dynabeads® 3 times with 1 mL PBS; add buffer, apply to a magnet for 2 min and remove the supernatant. Remove the tube from the magnet and repeat the wash twice.

* Incubation times as short as 10 min can be used with concentrated protein samples in volumes close to what was originally pipetted from the vial.

Efficient isolation of target molecules using Dynabeads® is dependent on the bead concentration, target-molecule concentration, the ligand's affinity for the target molecule and the specific binding kinetics involved. The Dynabeads® concentration will depend on the size of your specific molecule. Also the salt concentration and pH of the chosen binding, washing, and elution buffers can be varied depending on the type of molecule to be immobilized. Similarly, the selected buffer used in the downstream application should be optimized for the specific application. As the Dynabeads® M-270 Carboxylic Acid will not inhibit enzymatic activity, bead-bound material can be used directly in downstream analysis. Alternatively, the target molecule can be eluted off the Dynabeads® following conventional elution methods.

Washing of Coated Beads

All immobilization procedures require washing of the coated Dynabeads® to remove excess ligand and to block unreacted surface. **Note:** In order to quench the non-reacted activated carboxylic acid groups, incubate the Dynabeads® coated with ligand with either 50 mM Tris, pH 7.4 for 15 min or 50 mM ethanolamine in PBS, pH 8.0 for 60 min, at room temperature with slow tilt and rotation.

1. Wash the coated Dynabeads® a total of four times with 100 µL PBS or 50 mM Tris. Blocking protein like BSA or skimmed milk powder may be added to a concentration of 0.1–0.5% when it doesn't interfere with the downstream applications. Also 0.1% Tween®-20 or Triton X-100 can be added during washes to reduce non-specific binding.
2. Resuspend the coated Dynabeads® to the desired concentration in PBS or a Tris storage buffer.

Description of Materials

Dynabeads® M-270 Carboxylic Acid are uniform, monosized superparamagnetic beads coated with a hydrophilic layer of glycidyl ether, followed by further coating with carboxylic acid groups.

Related Products

Product	Cat. no.
DynaMag™-2	12321D
DynaMag™-5	12303D
HulaMixer® Sample Mixer	15920D
Dynabeads® M-280 Tosylactivated	14203
Dynabeads® M-270 Epoxy	14301
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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