invitrogen
by life technologies

Dynabeads® MyOne™ Carboxylic Acid

Catalog nos. 65011, 65012 **Store at 2°C to 8°C**

Pub. no. MAN0008462 **Rev. Date:** June 2013 (Rev. 8.0)

Product Contents

Cat. no.	Volume
65011	2 mL
65012	10 mL

Dynabeads® MyOne™ Carboxylic Acid contains 10 mg beads/mL, supplied in purified water.

Product Description

Dynabeads® MyOne™ Carboxylic Acid provides an excellent solid support for a wide range of biomagnetic separations, molecular manipulations and affinity isolations. Their size makes them particularly suitable for sample preparation and handling of nucleic acids and proteins.

A carbodiimide may be used for activation of Dynabeads® MyOne™ Carboxylic Acid for amide bonding with primary amines. Bifunctional cross-linkers may be used to introduce other functional groups like thiol, amine, maleimide, etc., onto the surface of the beads. This may be useful when coupling ligands which do not have available primary amine groups. Activation and coupling may be performed in organic solvents such as DMF or acetone. This will give better yields when introducing cross-linkers or low molecular weight ligands. Once coupled with a ligand, the magnetic beads can be added to a cell lysate or other suspensions containing the 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 beads are washed to give a pure sample. The magnetic beads do not inhibit enzymatic activity, and can be included directly in downstream analysis of the bead-bound target molecule. 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).
- Ligands.

General Guidelines

- Use a mixer that provides tilting and rotation of the tubes to ensure that the magnetic beads do not settle in the tube.
- Do not use this product with the MPC™-1 magnet (Cat. no. 12001D).
- Avoid air bubbles (foaming) during pipetting.
- Never use less than the recommended volume of magnetic beads.
- Carefully follow the recommended volumes and incubation times.

Washing and Blocking of Coated Dynabeads® Magnetic Beads

The washing steps included in protocols A and B are generally sufficient to remove any non-covalently bound ligand. If leakage of the ligand is observed, heat-stress during washing, or washing at higher pH may be required. Capping of the excess carboxylic acid groups or protein blocking after coupling is generally not needed. However, if further capping or blocking is required, follow these recommendations: a carboxylic acid group activated with a carbodiimide is very labile and will hydrolyse back to a carboxylic acid group if no reaction with an amine ligand has occurred. To achieve efficient capping or covalent coupling of a blocking reagent, it is important to add the reagent within 30 minutes after the activation.

Table 1: Recommended Buffers and Solutions

P15 mM MES, pH 6	0.32 g MES (2-[N-morpholino]ethane sulfonic acid, MW 213.25). Dissolve in 90 mL distilled water, adjust to pH 6 and adjust to 100 mL.
EDC (10 mg/mL)	10 mg EDC (N-Ethyl-N'-(3-dimethylaminopropyl) carbodiimide hydrochloride, MW 191.7). Dissolve in 1mL cold purified water immediately before use.
100 mM MES, pH 4.8	2.13 g MES (2-[N-morpholino]ethane sulfonic acid, MW 213.25). Dissolve in 90 mL distilled water, adjust to pH 4.8 and adjust to 100 mL.
1.25M EDC in (40 µL) in 100 mM MES pH 4.8	240 mg EDC (N-Ethyl-N'-(3-dimethylaminopropyl) carbodiimide hydrochloride, MW 191.7). Dissolve in 1 mL 100 mM MES pH 4.8 immediately before use.
TT Buffer	250 mL of 1 M Tris buffer pH 8 and 1 mL 10% Tween®-20 is diluted to 1000 mL with purified water.
TE Buffer	10 mL of 1 M Tris buffer pH 8 and 10 mL of 100 mM EDTA (Ethylenediaminetetraacetic acid disodium salt, MW 336.21) is diluted to 1000 mL with purified water.
PBS pH 7.4 (phosphate buffered saline)	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 to 1 litre.
PBS with 0.1% with BSA/HSA/ skimmed milk	Include 0.1% with BSA/HSA/skimmed milk (0.1 g) in 100 mL PBS (above).
PBS /Tween® 20/Triton® X-100	Include 0.5–1.0% Tween® 20/Triton® X-100 (50–100 mg) in 100 mL PBS (above). If a preservative is needed for storage of coated beads, a final concentration of up to 0.09% sodium azide may be added to the storage buffer. This preservative is cytotoxic and must be removed before use. **Caution:** Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

A method for slowing the reaction and allowing for a better blocking/capping is the addition of (Sulfo-) N-hydroxy Succinimide to generate NHS-ester intermediate on the beads. This is generally not very efficient with Dynabeads® Carboxylic Acid, but it may be an option in cases where it is established that the recommended coupling protocol results in a bead with too large excess un-reacted COOH groups. Activation with EDC and NHS should be done at pH 6, while coupling of ligand and blocking reagent can be performed at pH 6–8.

Isolate Target Molecule

Efficient isolation of target molecules using Dynabeads® magnetic beads is dependent on the bead-concentration, target molecule concentration, the ligand's affinity for the target molecule and the specific binding kinetics involved. The concentration of Dynabeads® magnetic beads depends 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 applications should be optimized for the specific application. The small size of these Dynabeads® magnetic beads (1 µm) presents a high surface area per mg magnetic beads and a corresponding high capacity for the target molecule. The effective binding capacity will depend on the size of the specific molecules to be immobilized. Because the Dynabeads® magnetic beads will not inhibit enzymatic activity, the bead-bound material can be used directly in downstream analysis. Alternatively, the target molecule can be eluted off the Dynabeads® magnetic beads following conventional elution methods.

Protocols

Activate and Couple Ligand to the Dynabeads® Magnetic Beads

The following protocols are examples of two common applications of Dynabeads® MyOne™ Carboxylic Acid; binding of antibodies (A) and binding of oligonucleotides (B). The protocols are scalable and can be optimized.

A. Bind antibodies (IgG)

- This protocol is based on 1 mL (10 mg) Dynabeads® magnetic beads, but can be directly scaled up.
- Use ~500 µg protein/mL Dynabeads® magnetic beads.
- The total volume during coupling should be adjusted so that the final Dynabeads® magnetic beads concentration is 20–50 mg/mL.
- The required amount of EDC varies depending on the performed coating procedure.

1. Resuspend the Dynabeads® magnetic beads by rolling the vial for > 30 minutes and transfer 1 mL to a new tube.
2. Place the tube in a magnet for 2 minutes and remove the supernatant.
3. Remove the tube from the magnet and add 1 mL 15 mM MES buffer pH 6.0, vortex for 5–10 seconds.
4. Place the tube on a magnet for 2 minutes and remove the supernatant.
5. Repeat steps 3–4 once.
6. Resuspend the Dynabeads® magnetic beads in 100 µL 15 mM MES buffer pH 6.0.
7. Add 100 µL EDC and incubate on a roller for 30 minutes at room temperature.
8. Place the tube in a magnet for 2 minutes and remove the supernatant*.
9. Add up to 400 µg of the antibody**, diluted in 15 mM MES buffer pH 6.0 to a total volume of 200–500 µL.
10. Incubate on a roller over night at room temperature.
11. Place the tube in a magnet for 2 minutes and remove the supernatant.
12. Remove the tube from the magnet and add 1 mL PBS with 0.1% Tween®-20; place the tube on a roller mixer for 10 minutes.
13. Place the tube on a magnet for 2 minutes and remove the supernatant.
14. Repeat steps 12–13 once.
15. Resuspend the magnetic beads in 200–500 µL PBS with 0.1% Tween®-20 and 0.1% BSA to give the wanted concentration. Add preservative as needed.

* Formation of an amide bond between a primary amino group of the ligand and the carboxylic acid groups on the surface of the Dynabeads® magnetic beads is mediated by carbodiimide activation. The intermediate product of the reaction between the carboxylic acid and the carbodiimide is very labile and will hydrolyze quickly. To get the desired immobilization of the ligand, it is therefore important to have the ligand immediately available, and proceed quickly from step 9 to step 10.

** The antibody solution should not contain BSA, Tris, or other substances which may react with the activated Dynabeads® magnetic beads. Add at least an equivalent amount of MES buffer to adjust the pH close to 6.0 for the best coupling result.

B. Bind oligonucleotides

- This protocol is based on 1 mL (10 mg) Dynabeads® magnetic beads, but can be directly scaled up.
- Use ~50 nmol oligonucleotides or peptides/mL Dynabeads® magnetic beads.
- The total volume during coupling should be adjusted so that the final Dynabeads® magnetic beads concentration is 20–50 mg/mL.
- Oligonucleotides can be coupled to the Dynabeads® magnetic beads via the 5' or 3' after amino (NH_2) modification by use of EDC to establish an amide bond.

1. Resuspend the Dynabeads® magnetic beads by rolling the vial for >30 minutes and transfer 1 mL to a new tube.
2. Place the tube in a magnet for 2 minutes and remove the supernatant.
3. Wash the Dynabeads® magnetic beads twice in 1 mL 100 mM MES buffer pH 4.8 and resuspend in 100 µL of the same buffer.
4. In a separate tube, mix 5'amine modified oligonucleotide (50 nmol) and 40 µL 1.25 M EDC in 100 mM MES pH 4.8, to a total volume of 100 µL.
5. Add the oligo/EDC solution to the Dynabeads® magnetic beads and mix by vortexing for 10 seconds.
6. Incubate the suspension on a roller mixer at room temperature ≥3 hours or overnight.
7. Wash the Dynabeads® magnetic beads 3 times with 1 mL TT buffer (each round incubated >30 minutes).
8. Resuspend the magnetic beads in TE buffer to 10 mg/mL or desired concentration.

Isolate Target Molecule

See "General Guidelines" for further info on isolation of target molecules.

Description of Materials

Dynabeads® MyOne™ Carboxylic Acid are 1 µm uniform, monosized superparamagnetic beads coated with carboxylic acid groups on the surface of the magnetic beads. The small Dynabeads® magnetic beads with the hydrophilic surface, ensures low non-specific binding, excellent dispersion abilities and easy handling in a wide variety of buffers. These beads also have a high magnetic mobility combined with low sedimentation rate making them ideal for automated assays.

Related Products

Product	Cat. no.
DynaMag™-2 Magnet	12321D
DynaMag™-5 Magnet	12303D
DynaMag™-15 Magnet	12301D
HulaMixer® Sample Mixer	15920D
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

REF on labels is the symbol for catalog number.

Limited Use Label License

The purchase of this product conveys to the purchaser the limited, nontransferable right to use the purchased amount of the product only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel. This product is for internal research purposes only and is not for use in commercial applications of any kind, including, without limitation, quality control and commercial services such as reporting the results of purchaser's activities for a fee or other form of consideration. For information on obtaining additional rights, please contact outlicensing@lifetech.com or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

Manufactured by Life Technologies AS, Norway. Life Technologies AS complies with the Quality System Standards ISO 9001:2008 and ISO 13485:2012.

Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at www.lifetechnologies.com/termsandconditions. If you have any questions, please contact Life Technologies at www.lifetechnologies.com/support.

SPEC-05779

For support visit www.lifetechnologies.com/support or email techsupport@lifetech.com

www.lifetechnologies.com

life technologies™