invitrogen
by life technologies

Dynabeads® M-280 Tosylactivated

Catalog nos. 14203, 14204 **Store at 2˚C to 8˚C**

Rev. Date: May 2012 (Rev. 010)

Product Contents

Cat. no	Volume
14203	2 mL
14204	10 mL

Dynabeads® M-280 Tosylactivated contains 30 mg beads/mL, supplied in purified water.

Product Description

Dynabeads® M-280 Tosylactivated are ideal for protein isolation due to their size and surface chemistry. Any ligand (e.g. antibody, protein, peptide or glycoprotein) containing amino or sulfhydryl groups can be covalently coupled to the bead surface. The beads are also suitable for separating cells and bacteria, as a solid phase in rapid immunoassays, for molecular applications or screening of phage display libraries. The efficacy of immunomagnetic separation is critically dependant on the specificity and avidity of the antibody or other protein ligand applied. A ligand protein with affinity for the specific target protein (e.g. markers, receptors, enzymes) can be coupled to the surface of Dynabeads® M-280 Tosylactivated.

The beads are added directly to the sample containing your target antibody/antigen. The beads bind to the target during a short incubation, and then the bead-bound target is separated by a magnet. Captured targets proteins may be eluted off the beads for further downstream analyses, or directly loaded on electrophoresis gels. The beads can be re-used several times after elution of the target protein, provided that the protein ligand is covalently coupled to the bead surface.

Required Materials

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

Buffers

See Table 1 for recommended buffers and solutions.

- Buffer A, B and C are used for pre-washing and coupling of Dynabeads® M-280 Tosylactivated. Buffer A is the recommended buffer for coupling. For pH labile ligands, use Buffer B. Do not add any protein (apart from your specific protein ligand), sugar etc. to these buffers.
- Buffer D and E are used for washing of all ligand-coupled beads. Buffer E can also be used for storage of ligand-coupled beads. Do not use these buffers or any buffer containing protein or aminogroups (e.g. glycine, Tris) for pre-washing or coupling to these beads. If BSA interferes with your downstream application, replace this with another protein (e.g. HSA) or a detergent (e.g. Tween® 20). Protein blocking is recommended as it reduces aggregation and non-specific binding. If a preservative is needed for the coupled beads, a final concentration of < 0.1% (w/v) sodium azide may be added to Buffer E.
  Caution: Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

General Guidelines

- The ligand must be free of any protein, sugar or stabilizer, as these will compete with the ligand for available binding sites.
- If you use less than recommended amounts of antibodies, you might reduce the potential binding capacity.
- Addition of ammonium sulphate (Buffer C) to a final concentration of 1.2 M (1.0–1.5 M) will increase the amount of antibody coupled to the beads, and can give an improved performance in some applications.
- Maximal covalent binding of ligand to the beads is achieved after 12–18 hours at 37°C. Coupling at 20°C requires a longer incubation time of >20 hours to achieve the same degree of chemical binding. At 4°C the chemical binding is very slow (>48 hours). Buffer A is recommended at low temperatures.

Table 1: Recommended Buffers and Solutions

Buffer A 0.1 M borate buffer pH 9.5	6.18 g H_3BO_3 (MW 61.83). Dissolve in 800 mL distilled water. Adjust pH to 9.5 using 5M NaOH and adjust volume to 1 L with distilled water.
Buffer B 0.1 M Na-phosphate buffer, pH 7.4	2.62 g $NaH_2PO_4 \times H_2O$ (MW 137.99) and 14.42 g $Na_2HPO_4 \times 2\ H_2O$ (MW 177.99). Adjust volume to 1 L with distilled water.
Buffer C 3 M ammonium sulphate in Buffer A or B	39.64 g $(NH4)_2SO^4$ dissolved in Buffer A or B. Adjust pH with NaOH or HCl. Adjust up to 100 mL with Buffer A or B.
Buffer D PBS pH 7.4 with 0.5% (w/v) BSA	Add 0.88 g NaCl (MW 58.4) and 0.5% (w/v) BSA to 80 mL 0.01 M sodium-phosphate pH 7.4. Mix thoroughly and adjust volume to 100 mL with 0.01 M sodium-phosphate pH 7.4.
Buffer E PBS pH 7.4 with 0.1% (w/v) BSA	Add 0.88 g NaCl (MW 58.4) and 0.1% (w/v) BSA to 80 mL 0.01 M sodium-phosphate pH 7.4. Mix thoroughly and adjust volume to 100 mL with 0.01 M sodium-phosphate pH 7.4.

Protein Isolations

Dynabeads® M-280 Tosylactivated can be used to separate different proteins. Efficient isolation of target proteins depends on bead concentration, target protein concentration, the affinity of the bead-coupled ligand to the target protein as well as the incubation time. Equilibrium binding of target to ligand will be reached after 5–60 min. Binding can be performed at 2°C to 37°C, please note that lower temperatures generally requires longer incubation times than higher temperatures. One mg of conjugated beads typically binds 1–10 µg target protein, but varies from application to application. Optimization is therefore required. If target proteins are present at very low concentrations, an increase in the amount of ligand-coupled beads is usually required.

Elution of Proteins

The target protein may be concentrated by elution in small volumes (down to 10 µL). 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 coupled onto the beads, target protein stability, downstream application and detection methods. To avoid eluting off non-specific binding to the tube walls, change tube before you elute. It is possible to reuse the ligand-coupled beads after mild elution. To ensure reuse of the ligand-coupled beads and functionality of the isolated target protein, return both beads and target protein to physiological pH (7.4) immediately after elution.

Use of Dynabeads® in Immunoassays

Dynabeads® M-280 Tosylactivated can be used as a solid phase in immunoassays (IA). Combining specific antigens or antibodies and the superparamagnetic properties of the beads ensure rapid reaction kinetics both in the binding process and in the separation of the analyte. The detection system can be based on the use of enzymes, radioisotopes, fluorescent substances or chemiluminescence. Couple Dynabeads® M-280 Tosylactivated with your assay-specific antigen or antibody. A careful titration of antigen or antibody, as well as a titration of the exact amount of beads per test is important for optimal reaction kinetics. Immunoassays using Dynabeads® M-280 Tosylactivated are based on several principles:

1. Couple the beads with an antigen and isolate specific antibodies against the antigen by a direct IA-method. Detection is done directly or indirectly with a secondary labeled antibody.
2. Couple the beads with one antibody (Ab1) and allow reaction with the antigen. A labeled antibody (Ab2) reacting with a different epitope on the antigen is used to detect the initial antigen-antibody complex. In a two-site IA ("sandwich" IA) there are two possible procedures; either incubate the antigen first with the antibody (Ab1) coupled to the bead surface and then with the labeled antibody (Ab2), or first incubate the antigen with the labeled antibody (Ab2) before incubating with the bead-antibody (Ab1) to form the complex.
3. Couple the beads with an antibody (Ab1) and allow reaction with the antigen and a labeled antigen in a competitive assay. Titrate the exact amount of beads for each IA-system. Depending on the antibodies used, assay conditions, detection system etc., 25–200 µg Ab-coupled beads per well (100 µL) is within normal range. Continuous mixing during the incubation of sample and beads is required. Reaction times of 10–60 min are generally sufficient. Determine the exact incubation time during your test optimization.

Protocols

Wash the Dynabeads®

See Table 2 for volume recommendation.

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 A or B, or at least 1 mL, and resuspend.
4. Place the tube in a magnet for 1 min and discard the supernatant.
5. Remove the tube from the magnet and resuspend the washed beads in the same volume of Buffer A or B as the initial volume of beads (step 2).

Couple Ligands to the Dynabeads®

- This protocol is based on 5 mg (~165 µL) Dynabeads® M -280 Tosylactivated. It is not recommended to couple < 5 mg beads at a time. For larger volumes than 10 mg beads, scale up all volumes accordingly, as shown in Table 2.
- Use 100 µg ligand/5 mg beads. The ligand volume (µL) is dependent on the ligand concentration (µg/uL) and must be calculated for each ligand, e.g. when coupling 5 mg beads, the Ab requirement is 100 µg. If the Ab concentration is 0.8 mg/mL you have to use 125 µL Ab (100 µg : 800 µg/mL = 0.125 mL).
- The optimal coupling concentration is ~40 mg beads/mL (step 4). It is decreased to 20 mg/mL when coupling the smallest amount (5 mg beads), to allow for a sufficient volume for efficient mixing.

1. Transfer 165 µL washed and resuspended beads to a new tube, place in a magnet for 1 min, and remove the supernatant.
2. Resuspend the beads in 100 µg ligand* and add Buffer A (or B) to give a total volume of 150 µL**. Mix thoroughly by vortex or pipetting.
3. Add 100 µL Buffer C and mix by vortexing or pipetting.
4. Incubate on a roller at 37°C for 12–18 hours.
5. Place the tube on a magnet for 2 min and remove the supernatant.
6. Remove the tube from the magnet and add 1 mL Buffer D, incubate at 37°C for 1 hour on a roller.
7. Place the tube on a magnet for 2 min and remove the supernatant.
8. Remove the tube from the magnet and add 1 mL Buffer E, vortex for 5–10 sec.
9. Place the tube on a magnet for 2 min and remove the supernatant.
10. Repeat steps 7–8 once.
11. Resuspend and dilute the beads in Buffer E to achieve your final desired bead concentration. See Table 2 for an example of 20 mg/mL.

Table 2: Volumes for ligand coupling to Dynabeads® M-280 Tosylactivated.

Step	Reagent volumes	5 mg beads	10 mg beads	50 mg beads
	Recommended tube	Micro centrifuge tube	Micro-centrifuge tube	Flow tube
	Recommended magnet	DynaMag™-2	DynaMag™-2	DynaMag™-15
1	Dynabeads® M-280 Tosylactivated	165 µL	335 µL	1675 uL
2*	Ligand	100 µg	200 µg	1000 µg
2**	Add Buffer A or B. Ligand + Buffer A/B volume should be:	150 µL	150 µL	750 µg
3	Buffer C	100 µL	100 µL	500 µL
***	Total coupling volume	250 µL	250 µL	1250 µL
6	Buffer D	~1 mL	~1 mL	~ 5 mL
8–10	Buffer E	2 × ~1 mL	2 × ~1 mL	2 × ~5 mL
11	Resuspend in Buffer E (end conc. 20 mg/mL)	240 µL	480 µL	2.4 mL

* Calculate the volume from the ligand (µg/mL) concentration.
** Note: If the ligand concentration is low, leave out the coupling buffer.
*** Optimal coupling concentration is 40 mg beads/mL, but can be reduced to 20 mg/mL if the coupling volume is low.

Isolate Target Protein

- One mg of conjugated beads will typically bind 1–10 µg target protein, but varies from application to application. Thus, optimization is required. If the target proteins are present at very low concentrations, an increase in the amount of ligand-coupled beads is usually required. Example: If your target protein has a conc. of 10 µg protein/mL, you will need 100–1000 µL.
- This protocol is based on 1 mg ligand-coupled beads as described. If you resuspended the beads to 20 mg/mL in step 11 above, 1 mg beads is equivalent to 50 µL.

1. Add 1-10 ug sample containing target protein to 50 uL of the ligand-coupled beads.
2. Incubate with tilting and rotation to capture the target protein.
3. Place the tube on the magnet for 2 min (longer for viscous samples). Pipet off the supernatant.
4. Remove the tube from the magnet and add 1 mL Buffer D or E, vortex for 5–10 sec.
5. Place the tube on a magnet for 2 min and remove the supernatant.
6. Repeat steps 4–5 twice.

Continue with e.g. elution or immunoassays. Se "General Guidelines" for more info.

Description of Materials

Dynabeads® M-280 Tosylactivated are 2.8 µm uniform, superparamagnetic, polystyrene beads coated with a polyurethane layer. These Dynabeads® bind proteins physically and chemically through primary amino or sulfhydryl groups, with an increasingnumber of covalent bonds with higher temperature and pH.

Related Products

Product	Cat. no.
DynaMag™-2	12321D
DynaMag™-5	12303D
DynaMag™-15	12301D
Dynabeads® MyOne™ Tosylactivated	65501
HulaMixer® Sample Mixer	15920D

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 13485:2003.

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-06017

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

www.lifetechnologies.com

life technologies™