invitrogen™ | DYNAL®
invitrogen bead separations

Cat. no. 142.03
142.04

Rev. no. 009

Dynabeads® M-280 Tosylactivated

For research use only

INDEX

1. PRODUCT DESCRIPTION

Materials

Product No.	Volume	Concentration
142.03	2 ml	30 mg/ml
142.04	10 ml	30 mg/ml

Dynabeads M-280 Tosylactivated are supplied in water.

Product Characteristics

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

Diameter: 2.8 µm
Surface area: 4–8 m^2/g
Active chemical functionality: 0.1–0.2 mmol/g
Density: 1.4 g/cm^3

Additional Materials Required

- Magnet (see www.invitrogen.com/magnets-selection for recommendations)
- Mixing/rotation device (e.g. HulaMixer™ Sample Mixer, Cat.no. 159.02D).
- Antibody/other selecting protein ligand
- Buffers/solutions (see below)

Recommended Buffers and Solutions

Coupling Buffers

Buffer A: 0.1M 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 litre with distilled water.

Buffer B: 0.1M Na-phosphate buffer pH 7.4
2.62 g $NaH_2PO_4 \times H_2O$ (MW 137.99) and 14.42 g $Na_2HPO_4 \times 2H_2O$ (MW 177.99) Dissolve in distilled water. Adjust to 1 litre.

Buffer C: 3M ammonium sulphate in Buffer A or B
39.64 g $(NH_4)_2SO_4$ dissolved in Buffer A or B. Adjust pH with NaOH or HCl. Adjust up to 100 ml with Buffer A or B.

- Buffer A and B are used for pre-washing and coupling of Dynabeads M-280 Tosylactivated.
- Do not add any protein (apart from your specific protein ligand), sugar etc. to these buffers. **Buffer A is the recommended buffer for coupling. For pH labile ligands, use Buffer B.**

Blocking Buffer

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 0.01M Na-phosphate pH 7.4. Mix thoroughly and adjust volume to 100 ml with 0.01M Na-phosphate pH 7.4.

Washing and Storage Buffer

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 0.01M Na-phosphate pH 7.4. Mix thoroughly and adjust volume to 100 ml with 0.01M Na-phosphate pH 7.4.

- Buffer D and E are used for washing of all ligand-coupled Dynabeads. Buffer E can be used for storage of ligand-coupled Dynabeads. Do not use these buffers or any buffer containing protein or amino-groups (glycine, Tris etc.) for pre-washing or coupling to Dynabeads M-280 Tosylactivated.
- If a preservative is needed for the coupled beads, a final concentration of < 0.1% (w/v) sodium azide (NaN_3) may be added to Buffer E. This preservative is cytotoxic and must be carefully removed from the beads by washing, before use. Required safety precautions must be followed when handling this material.
- If the presence of BSA will interfere with your downstream application, replace this protein in Buffers D and E 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.

2. INSTRUCTIONS FOR USE

2.1 Technical Advice

- The general protocol given below is based on experience with several antibodies and other protein ligands, and can be used as guidelines.

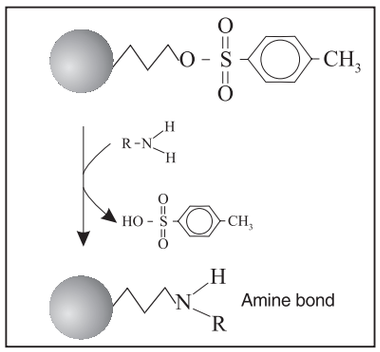
2.1.1 Protein ligand considerations

- A concentration of 20 µg antibodies per mg of Dynabeads is generally optimal (this corresponds to approximately 125 pmol IgG pr mg Dynabeads).
- The ligand to be coated on the beads 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 1.2M (1.0–1.5 M) will increase the amount of antibody coupled to the beads, and can give an improved performance in some applications.

2.1.2 Time/Temperature/pH

- Although physical adsorption to the bead surface is rapid, formation of covalent bonds requires additional time. Maximal covalent binding of ligand to the beads is achieved after the recommended 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.

2.2 Ligand Coupling Protocol



1. Resuspend the Dynabeads well. Transfer the required amount of Dynabeads (according to Table 1) to a new tube by pipetting.
2. Place the tube on a magnet, allow the beads to pellet completely (liquid will turn clear). Remove supernatant.
3. Remove the tube from the magnet.
4. Wash the beads by adding 1 ml of Buffer A or B, mix by vortexing or pipetting.
5. Place the tube on a magnet, allow the beads to pellet completely. Remove supernatant.
6. Remove the tube from the magnet.
7. Calculate the needed volumes of Buffer A (or Buffer B) and ligand, according to Table 1. Example for 5 mg of beads: Assuming an antibody concentration of 0.8 µg/µl, you will need to use 125 µl of antibody to reach the required 100 µg ligand. The final volume buffer + ligand needs to be 150 µl, therefore add 25 µl of Buffer A (or B). *NOTE: If, by this calculation, you need to use 150 µl of antibody, simply leave out the Coupling Buffer.*
8. Add the calculated amount of Buffer A (or B), and subsequently add the calculated amount of ligand to the beads. Mix by vortexing or pipetting.
9. Add Buffer C and mix by vortexing or pipetting.
10. Incubate on a roller at 37°C overnight (12–18 hours).
11. Place the tube on a magnet, allow the beads to pellet completely. Remove supernatant.
12. Remove the tube from the magnet.
13. Add 1 ml of Buffer D and incubate at 37°C for 1 hour on a roller.
14. Place the tube on a magnet, allow the beads to pellet completely. Remove supernatant.
15. Remove the tube from the magnet.
16. Add 1 ml Buffer E, vortex for 5–10 seconds.
17. Place the tube on a magnet, allow the beads to pellet completely. Remove supernatant.
18. Remove the tube from the magnet.
19. Repeat step 17–19.
20. Resuspend and dilute the beads in Buffer E to achieve your final desired bead concentration. See Table 1 for an example of 20 mg/ml.

Table 1: Recommendations for M-280 Tosylactivated

Beads (mg)	Beads (µl)	Ligand (µg)	Buffer A (or B) + ligand (µl)	Buffer C (µl)	Buffer E to give 20 mg/ml (µl)*
5	165	100	150	100	240
10	335	200	150	100	480
20	670	400	300	200	960
50	1675	1000	750	500	2400

* The volume of the beads have been included in the calculation, e.g. 5mg of beads have a volume of 10 µl.

2.3 Target Protein Isolation Protocol

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 minutes to 1 hour. Binding can be performed at 2–37°C, please note that lower temperatures generally requires longer incubation times than higher temperatures.

One mg of conjugated beads will typically bind 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 Dynabeads is usually required.

1. Add sample containing target protein to 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 to collect the beads at the tube wall. For viscous samples, increase the time on the magnet as needed. Pipette off the supernatant.
4. Wash the beads three times using e.g. PBS buffer.

5. 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. Examples of elution methods are 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.

2.4 Immunoassay

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 Dynabeads 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 (see section 2.2). A careful titration of antigen or antibody, as well as a titration of the exact amount of Dynabeads 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 labelled antibody.
2. Couple the beads with one antibody (Ab1) and allow reaction with the antigen. A labelled 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 labelled antibody (Ab2), or first incubate the antigen with the labelled 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 labelled antigen in a competitive assay. Remember to resuspend the Dynabeads M-280 Tosylactivated well by pipetting and vortexing for 1–2 min. Avoid foaming. 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 Dynabeads is required. Reaction times of 10–60 minutes are generally sufficient. Determine the exact incubation time during your test optimization.

3. GENERAL INFORMATION

Invitrogen Dynal AS complies with the Quality System Standards ISO 9001:2000 and ISO 13485:2003.

3.1 Storage and Stability

If stored unopened at 2–8°C the Dynabeads are stable until the expiration date stated on the label. These Dynabeads should not be autoclaved, but can be incubated with ethanol (70%, 1h) or gamma irradiated after freeze drying. Do not freeze these Dynabeads.
Dynabeads coupled with antibody can be stored at 4°C for several months without loss of target protein/antigen binding capacity. Wash ligand coupled Dynabeads once before use.
Avoid bacterial contamination of the ligand-coupled Dynabeads. If cytotoxic preservatives are added, remove these carefully by washing before use. Wash ligand-coupled Dynabeads stored for more than two weeks once for 5 min in PBS/BSA before use.

3.2 Technical Support

Please contact Invitrogen Dynal for further technical support (see contact details).
Certificate of Analysis/Compliance is available upon request.
The latest revision of the package insert/instruction for use is available on www.invitrogen.com.

3.3 Warnings and Limitations

This product is for research use only. Not intended for any animal or human therapeutic or diagnostic use. 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 build-up.
Material Safety Data Sheet (MSDS) is available at http://www.invitrogen.com.

3.4 Trademarks

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3.7 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.
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