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Dynabeads® MyOne™ Tosylactivated

Catalog nos. 65501, 65502 **Store at 2˚C to 8˚C**

Rev. Date: May 2012 (Rev. 004)

Product Contents

Cat. no.	Volume
65501	2 mL
65502	10 mL

Dynabeads® MyOne™ Tosylactivated contains 100 mg beads/mL, supplied in purified water.

Product Description

Dynabeads® MyOne™ Tosylactivated is a solid-phase designed for biomagnetic separations. Any ligand (e.g. antibody, protein, peptide or glycoprotein) containing amino or sulfhydryl groups can be covalently coupled to the surface of the beads.

Dynabeads® MyOne™ Tosylactivated needs to be coated with a ligand with affinity for e.g. the protein to be isolated. When added to a heterogeneous sample, the coated beads will bind to their target protein. After a short incubation, the sample is placed on a magnet for ligand separation.

Downstream Applications

The beads are widely used for protein purification due to their short separation times, flexible volumes, and gentle handling of proteins. The beads are also suitable as a solid phase in rapid immunoassays, for molecular applications or screening of phage display libraries and may also be used in separation of cells, bacteria and organelles.

- Protein Isolation
  Dynabeads® MyOne™ Tosylactivated can be used for protein purification after coating of the beads with a ligand with affinity for the protein to be isolated (markers, receptors, enzymes etc.). Immunoprecipitated proteins may be eluted off the beads for further downstream analyses, or directly loaded on electrophoresis gels. Provided that the ligand is covalently coupled to the bead-surface, the beads may be reused several times after elution of the target protein when using mild elution conditions (ligand dependent).
- Immunoassays
  Dynabeads® MyOne™ Tosylactivated are widely used as a solid phase in immunoassays. The combination of specific antigens or antibodies and the superparamagnetic properties of the Dynabeads® MyOne™ Tosylactivated provide rapid reaction kinetics both in the coating process, separation and during washing of the analyte. The specific properties of the Dynabeads® MyOne™ platform; the low sedimentation rate and improved reaction kinetics compared to Dynabeads® M-450, M-280 and M-270, makes them particularly suitable for automated immunoassays.
- Cell and Organelle Separation
  Dynabeads® MyOne™ Tosylactivated may also be used for cell and organelle separation, however, the larger Dynabeads® M-280 and M-450 product range is recommended for organelle and cell applications, due to their size and higher magnetic mobility. But, the beads are well suited for isolation of extremely fragile cells and organelles, due to their smaller size.

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/other selecting molecule.

Recommended Buffers/Solutions

- **Phosphate Buffered Saline (PBS) pH 7.4.**
- **Coating Buffer: 0.1 M sodium borate buffer pH 9.5:**
  6.183 g H_3BO_3 (MW 61.83).
  Dissolve in 800 mL distilled water.
  Adjust pH to 9.5 using 5 M NaOH and adjust volume to 1000 mL with distilled water.
  Note: The coating buffer is used for prewashing and coating of Dynabeads® MyOne™ Tosylactivated. Do not add any sugar, protein (apart from your ligand), i.e. to this buffer.
- **3 M ammonium sulphate (stock solution):**
  Preparation and handling shall be performed in a fume hood.
  39.6 g $(NH_4)_2SO_4$ (MW 132.1).
  Dissolve in 0.1 M sodium borate buffer (pH 9.5), control/adjust pH and adjust volume to 100 mL.
- **Blocking buffer: PBS pH 7.4 with 0.5% with BSA and 0.05% Tween® 20:**
  Blocking buffer is used for blocking of all pre-coated Dynabeads®. Do not use this buffer or any buffer containing protein or amino-groups (glycine, Tris etc.) for pre-washing or coating of Dynabeads® MyOne™ Tosylactivated.
- **Washing/storage buffer: PBS pH 7.4 with 0.1% with BSA and 0.05 Tween® 20:**
  For storage purposes, add 0.02% sodium azide, as a preservative.
  Caution: Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.
- **Elution buffers:**
  Any conventional method for protein elution can be used, e.g. 0.1 M citrate pH 3, 0.1 M glycine-HCl pH 2.5, or 0.1 M glycine-NaOH pH 10. All reagents used should be analytical grade.

General Guidelines

- Visit www.lifetechnologies.com/samplepreparation for recommended sample preparation procedures.
- Use a mixer that provides tilting and rotation of the tubes to ensure that Dynabeads® do not settle in the tube.
- This product should not be used with the MPC™-1 magnet (Cat. no. 12001D).
- Avoid air bubbles (foaming) during pipetting.
- Never use less than recommended volume of beads.
- Carefully follow the recommended volumes and incubation times.
- The coated beads can usually be stored for several months at 2°C to 8°C, depending on the stability of the immobilized ligand.

Antibody/Protein Optimalization

- The efficacy of immunomagnetic separation is critically dependant on the specificity and avidity of the antibody or other ligand applied.
- Ascites, hybridoma supernatants and commercially available antibodies should be checked for their content of active antibody fraction.
- Pre-treatment of antibody, acidification: In general, lowering pH to 2.5 for 15 min at room temperature or 1 hour at 1°C to 4°C, and then raising pH to approx. neutral prior to addition to the beads, will increase binding and function of antibodies, but this must be optimized for your ligand.

Time/Temperature/pH

- A maximal covalent binding is achieved after 16–24 hours at 37°C. Coating at room temperature will require >48 hours to obtain the same degree of chemical binding. Dynabeads® M-450 Epoxy or Dynabeads® M-270 Epoxy are better suited for chemical coupling at low temperatures.
- Both higher temperatures and a higher pH will speed up the formation of covalent bonds, provided that the ligand is stable/ functional under these conditions. Sodium borate buffer pH 9.5 is recommended. Molarities between 0.1 and 0.5 are optimal.

Elution of Target Protein

The target 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. See "Recommended Buffers/Solutions" section for elution buffer recommendations. The method of choice depends on affinity of target molecule to the ligand coated onto the beads, stability of the target molecule, downstream application and detection methods. It is possible to re-use the coated beads after mild elution. To ensure re-use of the coated beads and functionality of the isolated target molecule, bring both beads and target back to physiological pH (7.4) immediately after elution.

Use of Dynabeads® for Immunoassays

Dynabeads® MyOne™ Tosylactivated can be used as a solid phase in immunoassays (IA). The combination of specific antibody or antigen 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. With Dynabeads® MyOne™ Tosylactivated it is important to obtain optimal reaction kinetics and dynamic range. A careful titration of ligand, as well as a titration of the exact amount of Dynabeads® per test is important to obtain optimal reaction kinetics and dynamic range. Immunoassays using Dynabeads® MyOne™ Tosylactivated are based on several principles:

- Dynabeads® MyOne™ Tosylactivated are coated with an antigen and used to isolate specific antibodies against the antigen by a direct IA-method. Detection is done directly or indirectly by using a secondary labeled antibody.
- Dynabeads® MyOne™ Tosylactivated are coated with a capture antibody (Ab1) and allowed to react with the antigen. A labeled antibody (Ab2) reacting with a different epitope on the antigen is used for detection of the initial antigen-antibody complex. In a two-site IA ("sandwich" IA), there are two possible procedures. Either the antigen is first incubated with the antibody (Ab1) coupled to the bead surface and then with the labeled antibody (Ab2), or the antigen is first incubated with the labeled antibody (Ab2) before being incubated with the bead-antibody (Ab1) to form the complex.

- Dynabeads® MyOne™ Tosylactivated are coated with an antibody (Ab1) and allowed to react with the antigen and a labeled antigen in a competitive assay. The exact amount of beads for each IA system must be titrated and depends on the antibodies used, assay conditions etc. In general 0.1–1.0 mg coated beads per 100–500 µL sample volume is sufficient. Mixing improves kinetics, but the Dynabeads® MyOne™ will stay in suspension during the incubation of sample and beads. Reaction times of 10–60 min are generally sufficient.

Protocols

To obtain optimal results, you should optimize buffer conditions, amount of ligand, bead and ligand concentration, blocking agents, and overall process economy. Antibody/protein to be coated directly onto the surface of the beads must be purified, since all proteins will bind to the bead surface. Sugars or stabilizers may disturb the binding and should be removed from the antibody/protein preparation.

Wash the Dynabeads®

This protocol is based on 50 mg (500 µL) beads. If using larger volumes, scale up accordingly (see Table 1).

1. Resuspend the beads in the vial (i.e. vortex for >30 sec, or tilt and rotate for 5 min).
2. Transfer 500 µL beads to a tube.
3. Add 1 mL Coating Buffer 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 100 µL Coating Buffer.

Suggested Coating Protocol

- This protocol is for coating of protein/antibody to 50 mg (originally 500 µL, but concentrated to 100 µL in the washing process) Dynabeads® MyOne™ Tosylactivated.
- Use ~2 mg protein/antibody (~40 µg antibody/mg beads). Calculate the ligand volume from the concentration (µg ligand/mL).
- Bead concentration during coating: 40 mg/mL giving coupling volume of 1250 µL.

1. To 100 µL washed beads add coating buffer (735 µL minus the calculated ligand volume) and mix properly.
2. Add the calculated amount ligand and mix properly.
3. Add the calculated amount of 3 M ammonium sulphate stock solution (415 µL).
4. Incubate for 16–24 hours at 37°C with slow tilt rotation (do not let the beads settle during the incubation period).
5. Place the tube on the magnet for 2 min, and remove the supernatant.
6. Add the same total volume (1250 µL) of Blocking Buffer and incubate at 37 °C over night.
7. Place the tube on the magnet for 2 min and remove the supernatant.
8. Add 1 mL Washing/storage Buffer and resuspend.
9. Repeat steps 7–8 twice to give a total of 3 washes and resuspend the beads to the desired volume/concentration.

Protein Isolation Procedure

The ligand coupled Dynabeads® MyOne™ Tosylactivated can be used for the separation of different proteins. Efficient isolation of target molecules is dependent on bead concentration, target molecule concentration, the affinity of the ligand for the target molecule, and incubation time. Equilibrium target ligand binding is reached after 5 min to 1 hour. Binding is performed at a recommended bead concentration of 1–10 mg beads/ mL. The capacity of the coated beads will usually be in the range 1–20 µg target protein per mg beads, but will vary with the application. If the target is in very low concentration an increase in amount of coated beads is usually required. Optimize for your use.

1. Add sample containing target molecule to the coated beads.
2. Incubation with tilting and rotation to capture the target.
3. Place the tube on the magnet for 2 min (more for viscous samples). Pipet off the supernatant.
4. Wash the beads 3 times in PBS buffer. Optimization may be required.

Immunoassay

See "General Guidelines" for further info of Dynabeads® MyOne™ Tosylactivated used in IA.

Table 1: Volume overview for ligand coupling to Dynabeads® MyOne™ Tosylactivated.

Step	Reagent volumes	50 mg beads	200 mg beads
	Recommended tube	Microcentrifuge tube	Flow tube
	Recommended magnet	DynaMag™-2	DynaMag™-5
1*	Add beads	100 µL	400 µL
1	Add Coating Buffer (subtract the ligand volume)	735 µL – ligand volume	2940 µL – ligandvolume
2**	Add ligand volume	~2 mg	~8 mg
3	Add 3 M ammonium sulphate	415 µL	1660 µL
	Total coupling volume (40 mg beads/ml)	1250 µL	5 mL
6	Add Blocking Buffer	1250 µL	5 mL
7–9	Add Washing/storage Buffer	~1 mL × 3	~5 mL × 3

* Concentrated 5X from the original volume according to the "Wash the Beads" procedure.** Calculate the volume from the ligand (µg/mL) concentration. For storage, add the desired preservative and store at 2°C to 8°C.
Note: If the presence of BSA will interfere with your downstream application, this protein can be omitted from the buffer. Detergent may similarly be omitted.

Description of Materials

Dynabeads® MyOne™ Tosylactivated are 1 µm uniform, superparamagnetic, polystyrene beads coated with a polyurethane layer. The hydroxyl groups are activated by reaction with p-toluensulphonyl chloride. The resulting sulphonyl ester can subsequently react covalently with proteins or other ligands containing amino or sulfhydryl groups.

Related Products

Product	Cat. no.
DynaMag™-2	12321D
DynaMag™-5	12303D
DynaMag™-15	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	30212
Dynabeads® M-450 Epoxy	30202D
Dynabeads® MyOne™ Tosylactivated	65501
Dynabeads® MyOne™ Carboxylic Acid	65011

REF on labels is the symbol for catalog number.

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