invitrogen
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Dynabeads® M-450 Tosylactivated

Catalog no. 14013 **Store at 2˚C to 8˚C**

Rev. Date: June 2012 (Rev. 003)

Product Contents

Product contents	Volume
Dynabeads® M-450 Tosylactivated	5 mL

Product capacity

MNC: ~2 × 10^9 cells

Whole blood/buffy coat: ~200 mL

Dynabeads® M-450 Tosylactivated contains 4 × 10^8 beads/mL in distilled water.

Product Description

Dynabeads® M-450 Tosylactivated coupled with antibodies or other ligands provide a versatile tool for isolation of both cells and non-cell targets (e.g. proteins and other biomolecules). Their size makes them particularly suitable for stimulation and expansion of e.g. T cells. Cells can be directly isolated from any sample such as whole blood, bone marrow, mononuclear cell suspensions (MNC) or tissue digests

Dynabeads® M-450 Tosylactivated react covalently with proteins (e.g. antibodies) or other ligands containing primary amino or sulphydryl groups. Dynabeads® M-450 Tosylactivated bind proteins physically and chemically with an increasing number of covalent bonds with higher temperature and pH.

Once coupled with the specific ligand, the beads are mixed with the sample in a tube. The beads bind to the target during a short incubation, and then the bead-bound target is separated by a magnet.

Positive isolation – Discard the supernatant and use the bead-bound target for downstream applications.

Depletion – Discard the bead-bound target and use the remaining, untouched sample for downstream applications

Note: For protein purification and immunoassays we recommend using Dynabeads® M-280 Tosylactivated or Dynabeads® MyOne™ Tosylactivated.

Required Materials

- Magnet (DynaMag™ portfolio). See www.lifetechnologies.com/magnets for recommendations.
- Mixer allowing tilting and rotation of tubes (e.g. HulaMixer® Sample Mixer).
- **Buffer 1:** 0.1 M sodium phosphate buffer, pH 7.4–8.0 or 0.1 M sodium borate buffer, pH 7.6–9.5.
- **Buffer 2:** Ca^{2+} and Mg^{2+} free phosphate buffered saline (PBS) supplemented with 0.1% bovine serum albumin (BSA) and 2 mM EDTA, pH 7.4.
  Note: BSA can be replaced by human serum albumin (HSA) or fetal calf serum (FCS). EDTA can be replaced by sodium citrate.
- **Buffer 3:** 0.2 M Tris w/0.1% BSA, pH 8.5.
- Specific ligands.
- *Optional:* BSA for blocking and sodium azide as a preservative.
  Caution: Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

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 the beads do not settle in the tube.

Buffers

- Sugars and stabilizers may interfere with binding and should be removed from the ligand preparation.
- Coupling buffers should not contain any reactive groups (amines, thiols and hydroxyls) e.g. tris, glycine, or proteins.
- The pH and the ionic strength of the coupling buffer can be varied, but is generally carried out in 0.1 M phosphate buffer at pH 7.4–8.0. Tosyl groups are more reactive at higher pH, therefore 0.1 M borate buffer pH 7.6–9.5 can be used depending on the ligand stability. Buffers with higher ionic strength improve the coupling efficiency.

Coupling

The physical adsorption of the ligand to the bead surface is rapid, while the formation of covalent bonds will need more time. Maximal chemical binding is achieved after 16–24 hours at 37˚C. Coupling at 18˚C to 25˚C (room temperature) will require incubation time >48 hours to obtain the same degree of chemical binding. If a lower coupling temperature is required due to ligand stability, use Dynabeads® M-450 Epoxy.

Blocking

Adding a blocking protein such as 0.01–0.5% with BSA to the coupling solution may increase functionality of the coupled antibodies in cell isolation protocols. Blocking protein is generally added to coupling solution after 0–30 min. Both the incubation time before blocking and blocking protein concentration should be optimized.

Cell Isolation

- The efficiency of antibody-antigen binding can be increased for some applications by using a spacer molecule e.g. a secondary antibody coupled to the beads prior to coupling of the primary antibody.
- The optimal bead concentration during coupling is 4–8 × 10^8 beads per mL.
- The choice of primary antibody is the most important factor for successful cell isolation. Note that some antibodies may show reduced antigen-binding efficiency when coated onto beads, even though the antibody shows good results in other immunological assays.
- To avoid non-specific binding of cells (e.g. monocytes, B cells), add aggregated IgG to the cell sample to block Fc-receptors prior to adding the primary antibodies.
- When incubating beads and cells, the incubation temperature must be 2°C to 8°C to reduce phagocytic activity and other metabolic processes.
- Never use less than 25 µL (1 × 10^7) beads/mL cell sample and at least 4 beads per target cell.
- If the concentration of cells is increased or the target cell concentration exceeds 2.5 × 10^6 cells/mL, the bead volume must be increased accordingly. Cell concentration can be up to 1 × 10^8 cells/mL.
- Remove density gradient media (e.g. Ficoll): Wash cells prior to adding antibodies or beads.
- Serum may contain soluble factors (e.g. antibodies or cell surface antigens), which can interfere with the cell isolation protocol. Washing the cells once may reduce this interference.

Storage of Coupled Beads

- Coupled beads may typically be stored in Buffer 2 at 2°C to 8°C for months or even years. Stability of coupled beads depends on ligand stability and must be determined separately.
- A final concentration of 0.02% sodium azide can be added as a preservative to the storage buffer. The preservative must be removed by washing prior to cell isolation.

Release of Cells

Some cells exhibit high turnover of expressed antigens and will be released from the beads during 6–24 hours of incubation under standard culture conditions (37°C).

Protocols

Wash the Dynabeads®

See Table 1 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 1, 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 1 as the initial volume of beads (step 2).

Prepare Sample

- Cells can be directly isolated from any samples such as whole blood, bone marrow, MNC suspensions or tissue digests. See “General guidelines” for sample preparation.
- MNC should be re-suspended at 1 × 10^7 cells/mL in Buffer 2.

Couple Ligand to the Dynabeads®

This is a general procedure for antibody coupling to 1 mL (4×10^8) Dynabeads® M-450 Tosylactivated. It is not recommended to couple lower volumes than 200 µL beads. When coupling larger volumes, scale up all volumes accordingly, as shown in Table 1.

- Use approximately 200 µg antibody (Ab)/1 mL (4×10^8) beads.
- Calculate the Ab volume from the Ab concentration (µg/mL).

1. Transfer 1 mL of washed and resuspended beads to a tube.
2. Place the tube in a magnet for 1 min and discard the supernatant. Remove the tube from the magnet.
3. Resuspend the beads in Buffer 1 (1 mL minus antibody volume) and add 200 µg antibodies during mixing to reach a total coupling volume of 1 mL.
4. *Optional*: Incubate for 15 min and then add BSA to 0.01–0.1% w/v.
5. Incubate for 16–24 hours at room temperature with gentle tilting and rotation.
6. Place the tube in a magnet for 1 min and discard the supernatant.
7. Add 1 mL Buffer 2, mix and incubate for 5 min at 2°C to 8°C with gentle tilting and rotation. '
8. Apply to magnet for 1 min, remove the supernatant, remove from the magnet and repeat steps 7+8 once.
9. *Optional:* Incubate for 24 hours at room temperature or 4 hours at 37°C in 1 mL Buffer 3 to de-activate remaining free tosyl groups. Apply to magnet for 1 min and remove the supernata
10. Add 1 mL Buffer 2, mix and incubate for 5 min at 2°C to 8°C with gentle tilting and rotation.
11. Remove the beads from the magnet and resuspend the coated beads in 1 mL Buffer 2 (to obtain 4×10^8 beads/mL).

Table 1: Coupling of ligand to Dynabeads® M-450 Epoxy.

Step	Step description	Volumes per 4×10^8 beads	Volumes per 2×10^9 beads
	Recommended tube	Microcentrifuge tube	Flow tube
	Recommended magnet	DynaMag™-2	DynaMag™-5
1	Dynabeads® M-450 Tosylactivated	1 mL	5 mL
3	Resuspend the beads (Buffer 1)	1 mL minus ligand volume	5 mL minus ligand vol.
3	Add ligand (e.g. antibody)	Volume that equals 200 µg	Vol. that equals 1 mg
4	*Optional:* Add BSA	to 0.01 - 0.1% w/v	to 0.01 - 0.1% w/v
7+8*	Wash the beads (Buffer 2)	2 × ~1 mL	2 x ~5 mL
9*	*Optional:* De-activate tosyl groups (Buffer 3)	~1 mL	~5 mL
10*	Wash the beads (Buffer 2)	~1 mL	~5 mL
11	Resuspend the beads (Buffer 2)	1 mL	5 mL

* Adjust the Buffer volumes to fit to the tube you are using.

Isolate Cells

Use "Wash the Dynabeads®" procedure, replacing Buffer 1 with Buffer 2 to remove any soluble ligand prior to cell isolation. This protocol is based on 1×10^7 MNC or 1 mL whole blood, but is directly scalable from 1×10^7 to 4×10^8 cells or 1–40 mL whole blood. When working with volumes lower than 1×10^7 cells or 1 mL blood, use the same volumes as for 1×10^7 cells or 1 mL blood. When working with larger volumes, scale up all volumes accordingly, as shown in Table 2.

1. Add 25 µL pre-coupled, washed and resuspended beads to 1 mL prepared sample (1×10^7 MNC).
2. Incubate for 20 min (positive isolation) or 30 min (depletion) at 2°C to 8°C with gentle tilting and rotation.
3. *Optional:* Add 1 mL Buffer 2 to limit trapping of unbound cells.
4. Place the tube in a magnet for 2 min.
5. *For depletion:* Transfer the supernatant containing the unbound cells to a fresh tube for further experiments.
   or
   For positive isolation: Discard the supernatant and gently wash the bead-bound cells 4 times by:
   a. Add 1 mL Buffer 2.
   b. Place the tube in the magnet for 1 min and discard the supernatant. Remove the tube from the magnet.
   c. Repeat steps 6a-6b three times.
6. Resuspend the cells in buffer/medium for downstream application.

Table 2: Volumes for cell isolation.

Step	Step description	Volumes per 1×10^7 MNC	Volumes per 2×10^8 MNC
	Recommended tube	5–7 mL tubes	50 mL tubes
	Recommended magnet	DynaMag™-5	DynaMag™-50
1*	Bead volume	~25 µL	~500 µL
1	Cell volume (e.g. MNC, blood)	1 mL	20 mL
3**	Optional: Increase volume (Buffer 2)	~1 mL	~20 mL
5	**For positive isolation only:** Wash the cells (Buffer 2)	4 × ~1 mL	2 × ~20 mL

* If very high depletion-efficiency is required or you are depleting many cells simultaneously, you might have to increase/ optimize the amount of beads. ** Adjust the Buffer 2 volumes to fit to the tube you are using.

Description of Materials

Dynabeads® M-450 Epoxy are uniform, superparamagnetic polystyrene beads (4.5 µm diameter) covered with surface epoxy groups suitable for physical and chemical binding of antibodies and other bio-molecules.

Related Products

Product	Cat. no.
DynaMag™-2	12321D
DynaMag™-5	12303D
DynaMag™-15	12301D
DynaMag™-50	12302D
HulaMixer® Sample Mixer	15920D
Dynabeads® M-450 Epoxy	14011
Dynabeads® M-280 Tosylactivated	14203
Dynabeads® MyOne™ Tosylactivated	65501

REF on labels is the symbol for catalog number.

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