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Dynabeads® Mouse DC Enrichment Kit

Catalog no. 11429D **Store at 2˚C to 8˚C**

Rev. Date: March 2012 (Rev. 003)

Kit Contents

Kit contents	Volume
Depletion MyOne™ SA Dynabeads®	5 mL
Antibody Mix (for Mouse DC Kit)	1 mL

Kit capacity
~5 × 10^8 cells

Depletion MyOne™ SA Dynabeads® contains 10 mg beads/mL in phosphate buffered saline (PBS), pH 7.4, containing 0.1% bovine serum albumin (BSA) and 0.02% sodium azide as a preservative. The Antibody Mix contains a mixture of biotinylated monoclonal antibodies in PBS, pH 7.4, containing 0.02% sodium azide.
Caution: Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

Product Description

This product is intended for enrichment of untouched mouse dendritic cells (DCs) from mouse spleen or lymph node mononuclear cells (MNC) by depleting T cells, mIgM$^+$ B cells, NK cells, erythrocytes and most granulocytes. The DC enriched population is bead- and antibody-free, contains a very high recovery of Lin$^-$ CD11c$^+$ mouse cells, and is intended for further isolation of any DC sub-population by flow sorting.

Add a mixture of biotinylated monoclonal antibodies against non-DC cells to the starting sample to label the unwanted cells. Wash the cells and add Depletion MyOne™ SA Dynabeads® to bind to the antibody-labeled cells during a short incubation. Apply to a magnet and transfer the supernatant with the enriched DC's to a new tube and discard the bead-bound cells (fig. 1).

Downstream Applications

This kit provides high recovery of CD11c$^+$Lin$^-$ cells suitable for flow sorting to obtain any subpopulation, including DCs positive for CD4, CD8α, B220 and CD19.

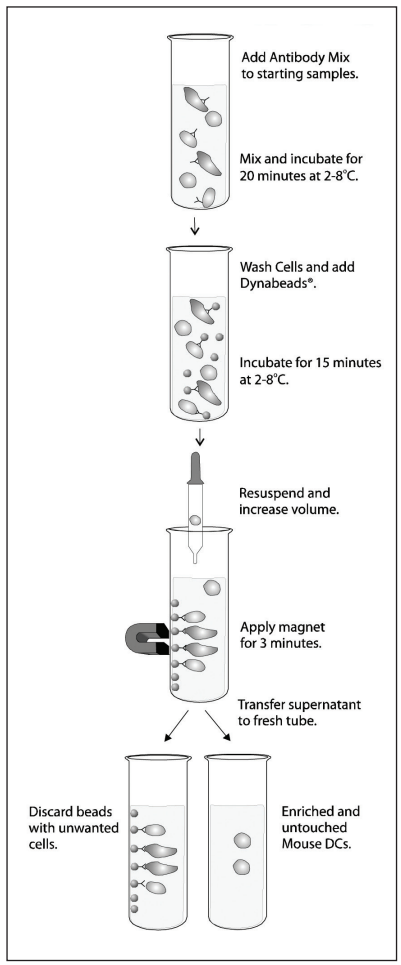

Figure 1: Simple method for enrichment of mouse DC's.

Required Materials

- Magnet (DynaMag™ portfolio). See www.lifetechnologies.com/magnets for recommendations.
- Mixing device with tilting and rotation, e.g. HulaMixer® Sample Mixer.
- Heat inactivated Fetal Bovine Serum (FBS)/Fetal Calf Serum (FCS).
- Buffer 1: PBS (Ca^{2+} and Mg^{2+} free) supplemented with 0.1% BSA and 2 mM EDTA.
  Note: BSA can be replaced by human serum albumin (HSA) or 2% FBS/FCS. EDTA can be replaced by 0.6% sodium citrate.
- Buffer 2: PBS pH 7.4 (Ca^{2+} and Mg^{2+} free).
- DNAse.
- 0.5 M EDTA.
- Density gradient medium, e.g. Nycodenz® 1.077 g/cm^3.
- Cell strainer, 70 µm.

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 beads do not settle in the tube.
- This product should not be used with the MPC™-1 magnet (Cat. no. 12001D).
- Follow the recommended volumes and incubation times.
- Avoid air bubbles (foaming) during pipetting.
- To secure a good recovery of enriched DCs, it is critical to keep the buffers cold.
- Do not use buffers or additives (i.e. FCS) containing biotin since this may reduce efficiency of depletion.

Protocol

Wash the Beads

See Table 1 for volume recommendations.

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 3 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 transferred of beads (step 2).

Prepare Sample

To increase cell purity we recommend running single cell suspensions of spleen or lymph node cells through a density gradient to remove platelets, cell debris and high density cells, as described in the following procedure. This protocol is based on isolation from a maximum of 5 spleens:

1. Remove the spleens or lymph nodes from recently killed mice and transfer to a 50 mL tube containing 30 mL Buffer 2 supplemented with 120 IU/mL DNAse at room temperature.
2. Crush the tissues through a 70 µm cell strainer into a new tube. Flush the cell strainer with the Buffer 2 to wash out all cells.
3. Incubate the tube for 15 min at room temperature with tilting and rotation.
4. Add 0.2 mL of 0.5 M EDTA to each tube to dissolve any DC-T cell aggregates.
5. Mix and incubate for 5 min on a roller at room temperature with tilting and rotation.

Perform further cell processing on ice with cold buffers.

6. Filter the cells through a new 70 µm cell strainer into a new 50 mL tube and flush the strainer with Buffer 1.
7. Fill the tube with Buffer 1.
8. Centrifuge at 350 × g for 10 min at 2°C to 8°C.
9. Discard the supernatant and resuspend the cell pellet in 5 mL Buffer 1.
10. Transfer the cell suspension and layer carefully on top of 4 mL density gradient medium in a 15 mL tube.
11. Centrifuge at 1700 × g for 10 min at 2°C to 8°C with slow acceleration and no brakes.
12. Carefully transfer the leucocyte layer to a new tube. Fill the tube with Buffer 1 and centrifuge at 350 × g for 10 min at 2°C to 8°C.

Count the cells and resuspend in Buffer 1 to a concentration of 1×10^8 cells/mL.

Enrich Mouse DCs

This protocol is based on 5×10^7 MNC, but it is scalable from 1×10^7– 3×10^8 cells, see Table 1. The protocol might need to be optimized if the cells are isolated from other sources than gradient centrifugation.

1. Transfer 500 µL (5×10^7) MNC in Buffer 1 to a tube.
2. Add 100 µL of Antibody Mix.
3. Mix well and incubate for 20 min at 2°C to 8°C.
4. Wash the cells by adding 10 mL Buffer 1. Mix well by tilting the tube several times and centrifuge at 300 × g for 10 min at 2°C to 8°C. Discard the supernatant.
5. Resuspend the cells in 4.5 mL Buffer 1.
6. Add 500 µL pre-washed and resupended Depletion MyOne™ SA Dynabeads®.
7. Incubate for 15 min at 2°C to 8°C with gentle tilting and rotation.
8. Add 5 mL Buffer 1.
9. Resuspend the bead-bound cells by gently pipetting 5 times using a pipette with a narrow tip opening. Avoid foaming.
10. Place the tube in the magnet for 3 min.
11. Transfer the supernatant containing the untouched DCs cells to a new tube.
12. *Optional:* Repeat steps 10–11 to remove any residual beads.

Table 1: Volumes for isolation of mouse DCs. This protocol is scalable from 1×10^7 to 3×10^8 leucocytes.

Step	Step description	Volumes per 5×10^7 leucocytes	Volumes per 3×10^8 MNC
	Recommended tube	15 mL tubes	50 mL tubes
	Recommended magnet	DynaMag™-15	DynaMag™-50
1	Cell volume	500 µL	3 mL
2	Antibody Mix for Mouse DC Kit	100 µL	600 µL
4*	Wash cells (Buffer 1)	~10 mL	~30 mL
5	Resuspend cells (Buffer 1)	4.5 mL	~27 mL
6**	Dynabeads® volume	500 µL	3 mL
9*	Volume added before magnet	~5 mL	~15 mL

* Adjust the Buffer 1 volumes to fit to the tube you are using.

** When incubating, tilt and rotate so the cells and beads are kept in the bottom of the tube. Do not perform end-over-end mixing if the volume is small relative to the tube size.

Description of Materials

Depletion MyOne™ SA Dynabeads® are uniform, superparamagnetic polystyrene beads (1.0 µm diameter) coated with streptavidin. The Antibody Mix for Mouse DC Kit contains an optimized mixture of biotinylated monoclonal antibodies against mouse CD2, CD3, CD49b, mIgM, and Ter-119.

Related Products

Product	Cat. no.
DynaMag™-5	12303D
DynaMag™-15	12301D
DynaMag™-50	12302D
HulaMixer® Sample Mixer	15920D

REF on labels is the symbol for catalog number.

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