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Dynabeads® Mouse CD43 (Untouched™ B Cells)

Catalog no. 11422D **Store at 2˚C to 8˚C**

Rev. Date: March 2012 (Rev. 002)

Product Contents

Product contents	Volume
Dynabeads® Mouse CD43 (Untouched™ B Cells)	5 mL

Product capacity
~2 × 10^9 cells

Dynabeads® Mouse CD43 (Untouched™ B Cells) contains ~40 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.
Caution: Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

Product Description

The product is intended for negative isolation of untouched resting mouse B cells by depletion of non-B cells from mouse spleen or lymph node cells. The isolated mouse B cells are pure, viable, and bead- and antibody-free, and can be used in any downstream application. The CD43 antibody on the surface of the beads will bind T cells, monocytes/macrophages, dendritic cells, NK- cells, granulocytes, platelets, and CD43 positive B cells (activated B cells, plasma cells, $CD5^+$ B1a cells).

Add Dynabeads® Mouse CD43 and allow the beads to bind the $CD43^+$ cells during a short incubation. Separate the bead-bound cells with a magnet. Collect the supernatant containing the untouched mouse B cells in a new tube and discard the bead-bound unwanted cells (fig. 1).

Downstream Applications

The untouched B cells can be used directly in a flow cytometer or used in any application, e.g. antigen presentation studies, analysis of B cell activation, proliferation and differentiation, B cell signaling pathway studies.

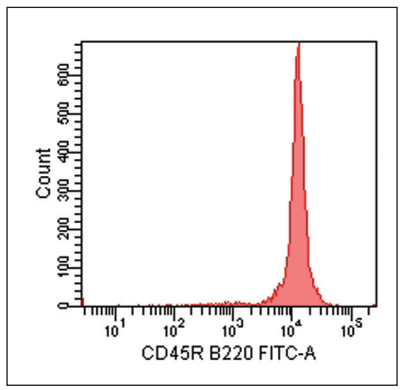

Figure 1: High purity (typically 98%) of mouse B cells after red cell lysis and isolation from mouse spleen with Dynabeads® Mouse CD43 (Untouched™ B Cells).

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).
- Isolation Buffer: 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.
- *Optional:* Red blood cell lysis buffer.
- *Optional:* For viability analysis, SYTOX® Red is recommended.

General Guidelines

- Visit www.lifetechnologies.com/samplepreparation for recommended sample preparation procedures.
- To secure a high recovery, it is important to pipette thoroughly (step 4 in "Isolate Untouched™ Mouse B Cells").
- To secure a high purity, lysis of red blood cells prior to isolation is recommended when working with spleen cells.
- 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.
- Keep the buffers cold.

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 Isolation Buffer, 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 Isolation Buffer as the initial volume transferred of beads (step 2).

Prepare Sample

- Prepare spleen or lymph node cells according to "General Guidelines"
- Resuspend the cells at 5 × 10^7 cells/mL in Isolation Buffer. The protocol might need to be optimized if the cells are isolated from other sources.

Isolate Untouched™ Mouse B Cells

This protocol is based on 5×10^7 leucocytes, but it is scalable from $1 \times 10^7 - 5 \times 10^8$ cells, according see Table 1.

1. Transfer 1 mL (5×10^7) cells in Isolation Buffer to a tube.
2. Add 125 µL of pre-washed and resuspended Dynabeads® Mouse CD43.
3. Mix well and incubate for 20 min at 18°C to 25°C with gentle tilting and rotation.
4. Resuspend the bead-bound cells by pipetting >10 times with a pipette with a narrow tip opening. Avoid foaming.
5. Add 2 mL Isolation Buffer (never add less than 1 mL even if working with smaller volumes) and resuspend.
6. Place the tube on the magnet for 2 min.
7. Transfer the supernatant containing the bead-free untouched mouse B cells to a new tube.
8. Repeat steps 6–7 to remove any residual beads.

Keep the cells on 2°C to 8°C until further use in downstream applications.

Table 1: Volumes for isolation of mouse B cells. This protocol is scalable from 1×10^7 to 5×10^8 cells

Step	Step description	Volume per 5×10^7 cells	Volume per 2×10^8 cells
	Recommended tube	5–7 mL	15 mL
	Recommended magnet	DynaMag™-5	DynaMag™-15
1	Cell volume	1 mL	4 mL
2*	Dynabeads® Mouse CD43	125 µL	500 µL
5**	Increase volume (Isolation Buffer)	~2 mL	~8 mL

* 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.

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

Description of Materials

Dynabeads® Mouse CD43 (Untouched™ B Cells) are uniform, superparamagnetic polystyrene beads (1 µm diameter) coated with streptavidin and conjugated to a biotinylated anti-mouse CD43 antibody.

Related Products

Product	Cat. no.
DynaMag™-5	12303D
DynaMag™-15	12301D
DynaMag™-50	12302D
HulaMixer® Sample Mixer	15920D
SYTOX® Red	S34859
Phosphate Buffered Saline	10010-023

REF on labels is the symbol for catalog number.

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