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# Dynabeads® Untouched™ Mouse CD8 Cells

**Catalog no. 11417D** **Store at 2˚C to 8˚C**

**Rev. Date:** March 2012 (Rev. 005)

## Kit Contents

| Kit contents | Volume |
|---|---|
| Mouse Depletion Dynabeads® | 2 × 10 mL |
| Antibody Mix (for Mouse CD8 cells) | 2 mL |

**Kit capacity**
~1 × $10^9$ cells

Mouse Depletion Dynabeads® contains 4 × $10^8$ 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 for mouse CD8 cells contains a mixture of rat monoclonal antibodies in PBS with 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 isolation of untouched mouse $CD8^+$ T cells from mouse spleen or lymph node cells by depleting $CD4^+$ T cells, B cells, monocytes/macrophages, NK cells, dendritic cells, erythrocytes, and granulocytes. Isolated untouched mouse $CD8^+$ T cells are bead- and antibody-free and suitable for use in any downstream application.

Add a mixture of monoclonal rat IgG antibodies against the non-$CD8^+$ T cells to the starting sample to label the unwanted cells.

Wash the cells and add Mouse Depletion Dynabeads® to bind to the antibody labeled cells during a short incubation.

Apply to magnet and transfer the supernatant with the untouched mouse $CD8^+$ T cells to a new tube and discard the bead-bound cells (fig. 1).

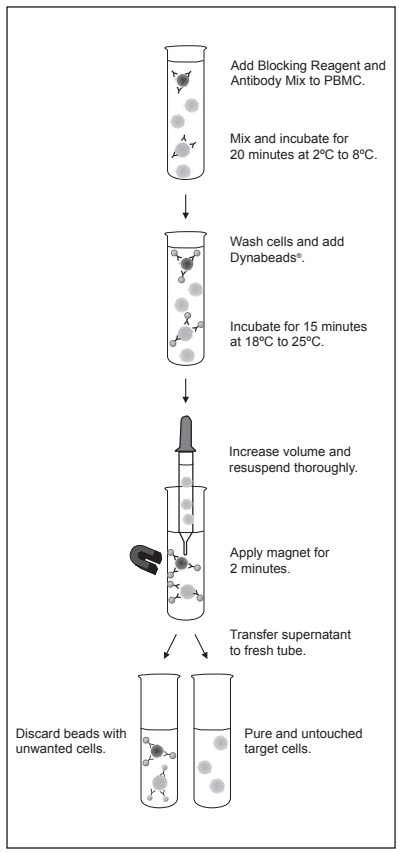


Figure 1: Simple method for isolating untouched mouse $CD8^+$ T cells.

## Downstream Applications

Isolated mouse $CD8^+$ T cells can be used in any application, (e.g., studies on $CD8^+$ T cell proliferation, cytotoxicity studies, studies on antigen-specific T cells, studies on regulation of $CD8^+$ T cell cytokine expression, flow cytometry/FACS sorting). Isolated cells can be activated/expanded using Dynabeads® Mouse T-Activator CD3/CD28 (polyclonal activation) or Dynabeads® Mouse T-Activator CD3/CD28/CD137 (antigen-specific activation).

## 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.

## 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.
- 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 1 × $10^8$ cells/mL in Isolation Buffer. The protocol might need to be optimized if the cells are isolated from other sources.

## Isolate Untouched Mouse CD8$^+$ Cells

This protocol is based on $5 \times 10^7$ leucocytes, but it is scalable from $1 \times 10^7 - 1 \times 10^9$ cells, see Table 1.

1. Transfer 500 µL ($5 \times 10^7$) leucocytes in Isolation Buffer to a tube.
2. Add 100 µL heat inactivated FCS/FBS.
3. Add 100 µL of Antibody Mix.
4. Mix well and incubate for 20 min at 2°C to 8°C.
5. Wash the cells by adding 10 mL Isolation Buffer. Mix well by tilting the tube several times and centrifuge at 350 × g for 8 min at 2°C to 8°C. Discard the supernatant.
6. Resuspend the cells in 4 mL Isolation Buffer.
7. Add 1 mL pre-washed and resuspended Mouse Depletion Dynabeads®.
8. Incubate for 15 min at 18°C to 25°C with gentle tilting and rotation.
9. Add 5 mL Isolation Buffer.
10. Resuspend the bead-bound cells by *gently* pipetting 5 times using a pipette with a narrow tip opening. Avoid foaming.
11. Place the tube in the magnet for 2 min and transfer the supernatant containing the untouched CD8$^+$ T cells to a new tube. Discard the beads with the unwanted cells.

Table 1: Volumes for isolation of mouse CD8$^+$ T cells. This protocol is scalable from $1 \times 10^7$ to $3 \times 10^8$ leucocytes.

| Step | Step description | Volumes per $5 \times 10^7$ leucocytes | Volumes per $3 \times 10^8$ leucocytes |
|---|---|---|---|
| | Recommended tube | 15 mL tubes | 50 mL tubes |
| | Recommended magnet | DynaMag™-15 | DynaMag™-50 |
| 1 | Cell volume | 500 µL | 3 mL |
| 2 | FCS/FBS | 100 µL | 600 µL |
| 3 | Antibody Mix for mouse CD8 cells | 100 µL | 600 µL |
| 5* | Wash cells (Isolation Buffer) | ~10 mL | ~30 mL |
| 6 | Resuspend cells (Isolation Buffer) | 4 mL | ~24 mL |
| 7** | Mouse Depletion Dynabeads® | 1 mL | 6 mL |
| 9* | Volume added before magnet | ~5 mL | ~15 mL |

* Adjust the Isolation Buffer 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

Mouse Depletion Dynabeads® are uniform, superparamagnetic polystyrene beads (4.5 µm diameter) coated with a polyclonal sheep anti-rat IgG antibody. The Antibody Mix for mouse CD8 cells contains a mixture of rat monoclonal antibodies against mouse CD4, CD45R (B220), CD11b (Mac1), Ter-119, and CD16/CD32.

## Related Products

| Product | Cat. no. |
|---|---|
| DynaMag™-5 | 12303D |
| DynaMag™-15 | 12301D |
| DynaMag™-50 | 12302D |
| HulaMixer® Sample Mixer | 15920D |
| Dynabeads® Mouse T-Activator CD3/CD28 | 11452D |
| Dynabeads® Mouse T-Activator CD3/CD28/CD137 | 11454D |
| Phosphate Buffered Saline | 10010-023 |

REF on labels is the symbol for catalog number.

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