

Dynabeads® Untouched™ Mouse CD4 Cells

Catalog nos. 11415D, 11416D

Store at 2°C to 8°C

Rev. Date: March 2012 (Rev. 006)

Kit Contents

Kit contents	11415D Volume	11416D Volume*
Mouse Depletion Dynabeads®	2 × 10 mL	4 mL
Antibody Mix (for Mouse CD4 cells)	2 mL	0.4 mL

* Sample size

Kit capacity

for 11415D: ~1 × 10⁹ cells

for 11416D*: ~2 × 10⁸ cells

Mouse Depletion Dynabeads® contains 4 × 10⁸ 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 CD4 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 CD4⁺ T cells from mouse spleen or lymph node cells by depleting CD8⁺ T cells, B cells, monocytes/macrophages, NK cells, dendritic cells, erythrocytes and granulocytes. Isolated untouched mouse CD4⁺ T cells are bead- and antibody-free, and suitable for use in any downstream application (fig. 1).

Add a mixture of monoclonal rat IgG antibodies against the non-CD4⁺ 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 CD4⁺ T cells to a new tube and discard the bead-bound cells.

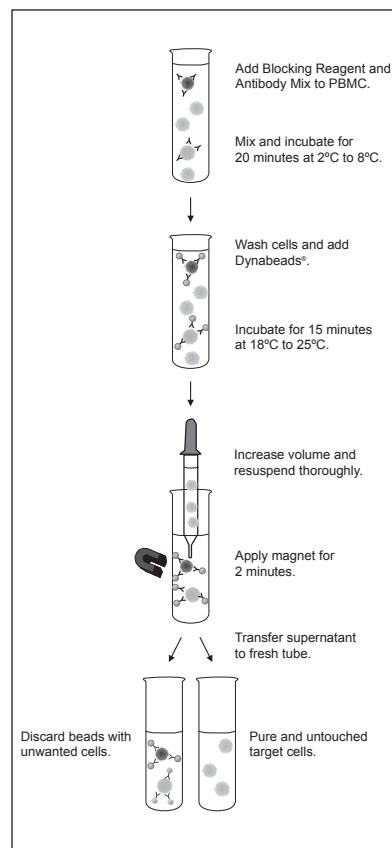


Figure 1: Simple method for isolating untouched mouse CD4⁺ T cells.

Downstream Applications

Isolated mouse CD4⁺ T cells can be used in any application, (e.g., studies on CD4⁺ T cell proliferation, apoptosis and induction of anergy, studies on antigen-specific T cells, studies on regulation of CD4⁺ 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 (Ca²⁺ and Mg²⁺ 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.

- Resuspend the beads in the vial (i.e. vortex for >30 sec, or tilt and rotate for 5 min).
- Transfer the desired volume of beads to a tube.
- Add the same volume of Isolation Buffer, or at least 1 mL, and resuspend.
- Place the tube in a magnet for 1 min and discard the supernatant.
- 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 × 10⁸ cells/mL in Isolation Buffer. The protocol might need to be optimized if the cells are isolated from other sources.

Isolate Untouched Mouse CD4⁺ Cells

This protocol is based on 5×10^7 leucocytes, but it is scalable from 1×10^7 – 1×10^9 cells, see Table 1.

1. Transfer 500 μ L (5×10^7) leucocytes in Isolation Buffer to a tube.
2. Add 100 μ L heat inactivated FCS/FBS.
3. Add 100 μ L 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 $350 \times g$ 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 CD4⁺ T cells to a new tube.

Table 1: Volumes for isolation of mouse CD4⁺ T cells. 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 leucocytes
	Recommended tube	15 mL tubes	50 mL tubes
	Recommended magnet	DynaMag™-15	DynaMag™-50
1	Cell volume	500 μ L	3 mL
2	FCS/FBS	100 μ L	600 μ L
3	Antibody Mix for mouse CD4 cells	100 μ L	600 μ L
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 μ m diameter) coated with a polyclonal sheep anti-rat IgG antibody. The Antibody Mix for mouse CD4 cells contains a mixture of rat monoclonal antibodies against mouse CD8, 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

REF on labels is the symbol for catalog number.

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