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Dynabeads® FlowComp™ Mouse CD4

Catalog no. 11461D **Store at 2˚C to 8˚C**

Rev. Date: December 2011 (Rev. 005)

Kit Contents

Kit contents	Volume
FlowComp™ Mouse CD4 antibody	1 mL
FlowComp™ Dynabeads®	3 mL
FlowComp™ Release Buffer	2 × 20 mL

Kit capacity
~2 × 10^9 cells

FlowComp™ Dynabeads® contains ~1 × 10^9 (~10 mg) beads/mL in phosphate buffered saline (PBS), pH 7.4, with 0.1% bovine serum albumin (BSA) and 0.02% sodium azide as a preservative. FlowComp™ Mouse CD4 antibody contains monoclonal anti-mouse CD4 antibody in PBS with 0.5% BSA and 0.02% sodium azide. FlowComp™ Release Buffer contains modified biotin in 0.1% BSA and 2 mM EDTA. **Caution:** Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

Product Description

This product is intended for positive magnetic isolation of $CD4^+$ T cells from lymphoid organs. The isolated cells are highly pure, viable, and bead-free (fig. 1). In the first step, FlowComp™ Mouse CD4 antibody is added and will bind to the target cells. In the second step, $CD4^+$ T cells that have bound the specific antibodies are captured by the Dynabeads®. In the third and last step, the cells are released from the Dynabeads®.

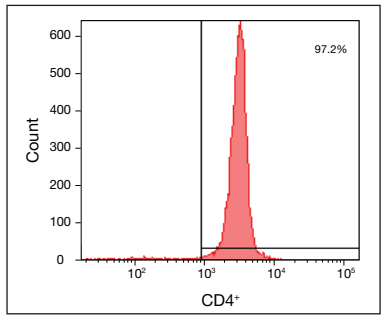

Figure 1: Mouse $CD4^+$ cells isolated with Dynabeads® FlowComp™ Mouse CD4

Downstream Applications

Isolated cells are bead-free and may be used directly in any downstream application including flow cytometry.

The cells readily proliferate in response to Dynabeads® Mouse T Activator CD3/CD28 and can be measured by incorporation of EdU or in a CFSE assay.

Required Materials

- Magnet (DynaMag™) See www.lifetechnologies.com/magnets for recommendations.
- Mixer allowing tilting and rotation of tubes (e.g. HulaMixer® Sample Mixer).
- Isolation Buffer: Ca^{2+} and Mg^{2+} free PBS supplemented with 0.1% BSA and 2 mM EDTA. **Note:** BSA can be replaced by human serum albumin (HSA) or 2% fetal bovine serum (FBS)/fetal calf serum (FCS).
- *Optional:* Flow cytometry antibodies. We recommend using anti-mouse CD4 R-PE (clone RM4-5) or anti-mouse CD3 Alexa Fluor® 488 as primary fluorescent antibody for flow staining of cells after isolation. Avoid using secondary antibodies specific for rat antibodies for flow cytometry staining.
- *Optional:* Red blood cell lysis buffer. See www.lifetechnologies.com/samplepreparation.
- *Optional:* For viability analysis, SYTOX® Red is recommended.

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 Dynabeads® do not settle at the bottom of the tube.
- This product should not be used with magnet MPC™-1.
- Follow the recommended volumes and incubation times (Table 1).
- Avoid air bubbles (foaming) during pipetting.
- To avoid unspecific labeling of cells during flow staining we recommend using gamma-globulin or Fc blocking reagents prior to staining with primary fluorescent antibody.
- Only DSB-X biotinylated antibodies can be used in the isolation process. Standard biotinylated antibodies will not give release of cells after isolation.

Protocol

Approximately 30–35% of mouse spleen cells are T cells, and about 70% of these T cells strongly express the CD4 antigen. This kit isolates highly pure $CD4^+$ T cells without contamination of CD4 expressing monocytes using Dynabeads® FlowComp™ Mouse CD4.

Prepare Cells

- Prepare a single cell suspension from lymphoid organs (e.g. lymph nodes or spleen) according to "General Guidelines".
- Resuspend the cells at 1 × 10^8 cells/mL in Isolation Buffer.
- Prepare approximately 10 mL of Isolation Buffer per 5 × 10^7 cells.

Wash Dynabeads®

See Table 1 for volume recommendations.

1. Resuspend the Dynabeads® in the vial (i.e. vortex for >30 sec, or tilt and rotate for 5 min).
2. Transfer the desired volume of Dynabeads® 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 Dynabeads® in the same volume of Isolation Buffer as the initial volume of Dynabeads® (step 2).

Isolate Cells

This protocol is based on 5×10^7 cells, but is directly scalable from 1×10^7 to 5×10^8 cells, according to Table 1. When working with fewer cells than 1×10^7, use the same volumes as for 1×10^7.

1. Transfer 500 µL (5×10^7) prepared cells to a tube and add 25 µL FlowComp™ Mouse CD4 antibody.
2. Mix well and incubate 10 min at 2°C to 8°C.
3. Wash by adding 2 mL Isolation Buffer and centrifuge 8 min at 350 × g.
4. Remove the supernatant, and resuspend in 1 mL Isolation Buffer.
5. Add 75 µL washed FlowComp™ Dynabeads® and mix well (e.g. vortex 2–3 seconds).
6. Incubate for 15 min at 2°C to 8°C under rolling and tilting.
7. Add 1 mL Isolation Buffer, pipet 2–3 times (or vortex 2–3 seconds) and place the tube in a magnet for 2 min.
8. While the tube is still in the magnet, carefully remove and discard the supernatant containing the CD4 negative cells.
9. Repeat steps 7–8 at least once to wash the bead-bound $CD4^+$ cells. These steps are critical to obtain a high purity of isolated cells.

Release Cells

10. Resuspend the bead-bound cells in 1 mL Release Buffer.
11. Incubate 10 min with rolling and tilting at room temperature.
12. Pipet 10 times to efficiently release the cells and place in a magnet for 2 min. Avoid foaming.
13. Transfer the supernatant containing the bead-free $CD4^+$ cells to a new tube and again place on the magnet for 1 min to remove any residual beads. Transfer again the supernatant containing the bead-free cells to a new tube.
14. Add 2 mL Isolation Buffer followed by centrifugation for 8 min at 350 × g. Discard the supernatant and resuspend the cell pellet in preferred cell medium.

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

Table 1: Volumes for mouse $CD4^+$ T cells. This protocol is scalable from 1×10^7 to 5×10^8 cells.

Step	Step description	Volumes per 5×10^7 cells	Volumes per 5×10^8 cells
	Recommended tube size	5 mL	15 mL
	Recommended magnet	DynaMag™-5	DynaMag™-15
1	Cell volume	500 µL	5 mL
1	FlowComp™ Human CD4 antibody	25 µL	250 µL
3*	Wash cells (Isolation Buffer)	~2 mL	~10 mL
4	Resuspend cells (Isolation Buffer)	1 mL	10 mL
5**	FlowComp™ Dynabeads®	75 µL	750 µL
7+9	Wash beads (Isolation Buffer)	2 × 1 mL	2 × 10 mL
10	FlowComp™ Release Buffer	1 mL	10 mL
14*	Wash cells (Isolation Buffer)	~2 mL	~20 mL

* Adjust the Isolation Buffer volumes to fit to the tube you are using. For very large volumes use a larger tube than recommended in step 14 to successfully remove the biotin in the sample.

** When incubating, tilt and rotate the vial 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

Dynabeads® FlowComp™ are uniform, superparamagnetic polystyrene beads (2.8 µm in diameter) coated with modified streptavidin. FlowComp™ Mouse CD4 antibody contains a DSB-X conjugated monoclonal rat anti-mouse IgG2a CD4. FlowComp™ Release Buffer contains a modified biotin that out-competes the modified biotin on the antibody to give the cell release from the beads.

Related Products

Product	Cat. no.
DynaMag™-5	12303D
DynaMag™-15	12301D
DynaMag™-50	12302D
HulaMixer® Sample Mixer	15920D
Rat anti-mouse CD4 R-P	MCD0404
Hamster anti-mouse CD3 Alexa Fluor® 488	HM3420
Dynabeads® Mouse T-Activator CD3/CD28	11452D
Phosphate buffered saline	14190
SYTOX® Red	S34859

REF on labels is the symbol for catalog number.

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