invitrogen
by life technologies

Dynabeads® FlowComp™ Mouse CD49b

Catalog no. 11464D **Store at 2˚C to 8˚C**

Rev. Date: December 2011 (Rev. 002)

Kit Contents

Kit contents	Volume
FlowComp™ Mouse CD49b 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 CD49b antibody contains monoclonal anti-mouse CD49b 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 CD49b$^+$ NK cells from lymphoid organs. The isolated cells are highly pure, viable, and bead-free (fig. 1). In the first step, FlowComp™ Mouse CD49b antibody is added and will bind to the target cells. In the second step, CD49b$^+$ NK 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®.

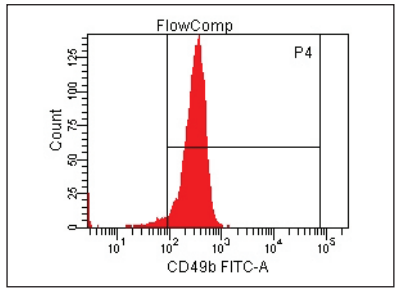

Figure 1: Purity of isolated CD49b+ NK cells after isolation. Dynabeads® FlowComp™ MouseCD49b were typically above 80% pure CD49b cells.

Downstream Applications

Isolated cells are bead-free and may be directly analyzed by flow cytometry and used in any downstream application such as expansion or cytotoxicity assays (i.e. Cr-release and LAMP-1 expression).

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:* We recommend using anti-mouse CD49b clone Ha1/29 as primary fluorescent antibody. Other clones might be blocked for optimal binding to the isolated cells. 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 6–8% of mouse spleen cells express CD49b, including most subsets of NK cells, subsets of NKT cells, TCR T cells, and some myeloid cells. This protocol describes isolation of highly pure CD49b$^+$ NK cells from 5 × 10^7 mouse splenocytes using Dynabeads® FlowComp™ Mouse CD49b.

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 CD49b 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 CD49b negative cells.
9. Repeat steps 7–8 at least once to wash the bead-bound CD49b$^+$ 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 20 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 CD49b$^+$ 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 CD49b$^+$ 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 CD49b antibody	25 µL	250 µL
3*	Wash cells (Isolation Buffer)	~2 mL	~10 mL
4	Resuspend cells (Isolation Buffer)	0.5 mL	5 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 (1.0 µm in diameter) coated with modified streptavidin. FlowComp™ Mouse CD49b antibody contains a DSB-X conjugated monoclonal rat anti-mouse IgM CD90.2 that is expressed on most subsets of NK cells, subsets of NKT cells, TCR T cells and some myeloid cells. CD49b is expressed by most commonly used inbred mouse strains, such as BALB/c and C57Bl/6. 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
Phosphate buffered saline	14190
SYTOX® Red	S34859

REF on labels is the symbol for catalog number.

Limited Use Label License

The purchase of this product conveys to the purchaser the limited, nontransferable right to use the purchased amount of the product only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel. This product is for internal research purposes only and is not for use in commercial applications of any kind, including, without limitation, quality control and commercial services such as reporting the results of purchaser's activities for a fee or other form of consideration. For information on obtaining additional rights, please contact outlicensing@lifetech.com or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

Manufactured by Life Technologies AS, Norway. Life Technologies AS complies with the Quality System Standards ISO 9001:2008 and ISO 13485:2003.

Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at www.lifetechnologies.com/termsandconditions. If you have any questions, please contact Life Technologies at www.lifetechnologies.com/support.

SPEC-06421

For support visit www.lifetechnologies.com/support or email techsupport@lifetech.com

www.lifetechnologies.com

life technologies™