invitrogen
by life technologies

Dynabeads® FlowComp™ Human CD3

Isolation directly from whole blood

Catalog no. 11365D **Store at 2˚C to 8˚C**

Rev. Date: February 2012 (Rev. 001)

Kit Contents

Kit contents	Volume
FlowComp™ Human CD3 Antibody	1 mL
FlowComp™ Dynabeads®	3 mL
FlowComp™ Release Buffer	2 × 20 mL

Kit capacity
Whole blood: 80 mL

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™ Human CD3 Antibody contains monoclonal CD3 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

Dynabeads® FlowComp™ Human CD3 positive magnetic isolation of $CD3^+$ T cells directly from anti-coagulated whole blood. Thus no sample preparation (e.g. density gradient centrifugation or lysis of red blood cells) is required. The isolated cells are highly pure, viable, and bead-free (fig. 1). In the first step, FlowComp™ Human CD3 Antibody is added and binds to the target cells. In the second step, $CD3^+$ T cells, that have bound the specific antibodies, are captured by the FlowComp™ Dynabeads®. In the third and last step, the cells are released from the FlowComp™ Dynabeads®.

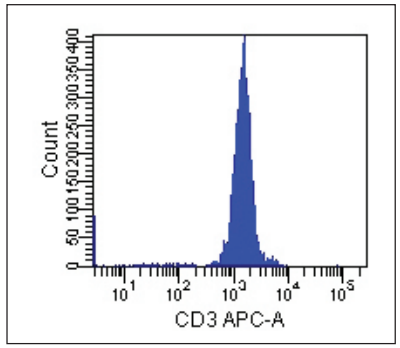

Figure 1: Purity of human $CD3^+$ cells isolated from whole blood using Dynabeads® FlowComp™ Human CD3.

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® Human T-Activator CD3/CD28 and can be measured by incorporating EdU or in a CFSE assay.

Required Materials

- Magnet (DynaMag™ portfolio). 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% FBS/FCS.
- *Optional:* Flow cytometry antibodies. We recommend using anti-CD3 clone UCHT-1 from Caltag Medsystems as primary fluorescent antibody for flow staining of cells after isolation. Optional clones: OKT3, HITa3.
- *Optional:* For viability analysis, SYTOX® Red is recommended

General Guidelines

- Use a mixer that provides tilting and rotation of the tubes to ensure that beads do not settle in the tube.
- Avoid spilling of sample in tube cap during rotating and tilting. If so, change tube cap. We recommend raising one end of the rotator during incubation.
- This product should not be used with the MPC™-1 magnet (Cat. no. 12001D).
- Avoid air bubbles (foaming) during pipetting.
- Never use less than the recommended volume of beads.
- Carefully follow the recommended pipetting volumes and incubation times.
- Keep all buffers cold.
- To avoid unspecific labeling of cells during flow staining, we recommend using gammaglobulin prior to staining with primary fluorescent antibody.
- For better purity, repeat the washing step once or transfer the bead-bound cells to a new tube before adding the FlowComp™ Release Buffer
- All incubations at room temperature can also be performed at 2°C to 8°C.

Protocol

In human whole blood from normal blood donors, approximately 15–20% of all leucocytes express CD3. This protocol describes magnetic capture and isolation of highly pure $CD3^+$ T cells from 2 mL of whole blood using Dynabeads® FlowComp™ Human CD3. The protocol is scalable. When working with blood volumes >2 mL, scale up all volumes accordingly, as shown in Table 1.

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 from step 2, 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 of beads (step 2).

Prepare Cells

- Collect whole blood sample in a collection tube containing an appropriate anticoagulant, (e.g. EDTA, heparin, ACD, or citrate).
- To avoid internalization of the CD3 molecule, cool the blood on ice to 2°C to 8°C prior to isolation.
- Prepare approximately 20 mL of isolation buffer per 2 mL whole blood.

Isolate Cells

This protocol is based on 2 mL whole blood, but is scalable according to Table 1.

1. Transfer 2 mL pre-cooled anti-coagulated whole blood to a tube on ice and add 25 µL FlowComp™ Human CD3 Antibody.
2. Mix well and incubate for 10 min on ice.
3. Add 4 mL Isolation Buffer and mix well, followed by centrifugation for 15 min at 350 × g with no brakes.
4. Aspirate the supernatant and discard the 4 mL volume added in step 3, (but keep at least 1 cm above cell pellet to avoid losing leucocytes).
5. Add 75 µL resuspended FlowComp™ Dynabeads® and mix well by vortexing.
6. Incubate for 15 min at room temperature under rolling and tilting.
7. Add 4 mL Isolation Buffer, mix well (or vortex 2–3 sec) and place the tube in the magnet for minimum 3 min.
8. While the tube is still in the magnet, carefully remove and discard the supernatant containing the CD3 negative cells.
9. Repeat steps 7–8 twice to wash the bead-bound $CD3^+$ cells. These steps are critical to obtain a high purity of isolated cells.

Release Cells

10. Resuspend in 1 mL FlowComp™ Release Buffer and pipet 3–4 times.
11. Incubate 10 min at room temperature under rolling and tilting.
12. Pipet 10 times to efficiently release the cells and place in a magnet for 1 min. Avoid foaming.
13. Transfer the supernatant containing the bead-free 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 human $CD3^+$ T cells. This protocol is scalable from 2–25 mL whole blood.

Step	Step description	Volumes per 2 mL whole blood	Volumes per 20 mL whole blood
	Recommended tube size	5 mL	50 mL
	Recommended magnet	DynaMag™-5	DynaMag™-50
1	Whole blood	2 mL	20 mL
1	FlowComp™ Human CD3 Antibody	25 µL	250 µL
3	Wash cells (Isolation Buffer)	4 mL	40 mL
5*	FlowComp™ Dynabeads®	75 µL	750 µL
7–9	Wash beads (Isolation Buffer)	3 x 4 mL	3 x 40 mL
10	FlowComp™ Release Buffer	1 mL	10 mL
14	Wash cells (Isolation Buffer)	2 mL	20 mL

* 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

FlowComp™ Dynabeads® are uniform, superparamagnetic polystyrene beads (2.8 µm in diameter) coated with modified streptavidin. FlowComp™ Human CD3 Antibody contains a DSB-X conjugated monoclonal mouse anti-human CD3. FlowComp™ Release Buffer contains a modified biotin that displaces the modified biotin on the antibody to release cells from the beads.

Related Products

Product	Cat. no.
DynaMag™-5	12303D
DynaMag™-15	12301D
DynaMag™-50	12302D
HulaMixer® Sample Mixer	15920D
Dynabeads® Human T-Activator CD3/CD28	11131D
Phosphate buffered saline	14190
SYTOX® Red	S34859
Click-iT®-EdU	A10202
CFSE assay	C34554

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-06645

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

www.lifetechnologies.com

life technologies™