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Dynabeads® FlowComp™ Human CD14

Isolation directly from PBMC

Catalog no. 11367D **Store at 2˚C to 8˚C**

Rev. Date: February 2012 (Rev. 001)

Kit Contents

Kit contents	Volume
FlowComp™ Human CD14 Antibody	1 mL
FlowComp™ Dynabeads®	2 × 3 mL
FlowComp™ Release Buffer	2 × 20 mL

Kit capacity
PBMC: ~2 × 10^9

FlowComp™ Dynabeads® contains ~1.5 × 10^9 beads (15 mg)/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 CD14 Antibody contains monoclonal CD14 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 CD14 is intended for positive isolation of $CD14^+$ monocytes from Peripheral Blood Mononuclear Cells (PBMC). The isolated cells are highly pure, viable, and bead-free (fig. 1). For isolation directly from buffy coat or whole blood, see the separate protocol supplied. In the first step, FlowComp™ Human CD14 Antibody is added and binds to the target cells. In the second step, $CD14^+$ 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®.

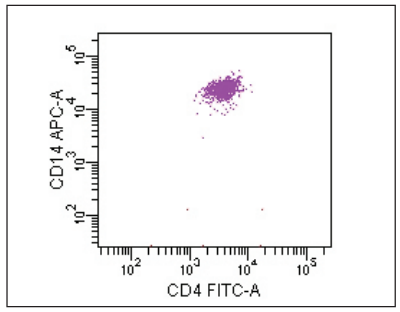

Figure 1: Purity of human $CD14^+$ cells isolated from PBMC using Dynabeads® FlowComp™ Human CD14.

Downstream Applications

Isolated cells are bead-free and can be used for flow cytometric analysis, cell culture, functional assay, or differentiation into monocyte-derived dendritic cells.

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% fetal bovine serum (FBS)/fetal calf serum (FCS).
- *Optional:* Flow cytometry anti-bodies. We recommend using anti-CD14 clone Tuk4 as a primary fluorescent antibody for flow staining of cells after isolation.
- *Optional:* For viability analysis, SYTOX® Red is recommended.

General Guidelines

- It is especially important to keep all buffers cold when working with monocytes.
- Visit www.lifetechnologies.com/cellisolation and follow our QuickLinks 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).
- Avoid air bubbles (foaming) during pipetting.
- Never use less than the recommended volume of beads.
- Carefully follow the recommended pipetting volumes and incubation times.
- 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.

Protocol

This protocol is intended for isolation of bead-free $CD14^+$ monocytes starting with 5 × 10^7 PBMC/test where typically 10–20% are $CD14^+$ monocytes. When working with higher cell numbers/number of tests, 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

- Prepare a PBMC suspension 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.
- To avoid monocytes phagocytosing the beads, which will lead to reduced monocyte recovery, *it is important to keep all buffers and reagents cold (2°C to 8°C) during the entire isolation procedure.*

Isolate Cells

This protocol is based on 5×10^7 PBMC, but is directly scalable from 1×10^7 to 5×10^8 cells, according to Table 1.

1. Transfer 500 µL (5×10^7) prepared cells to a tube and add 25 µL FlowComp™ Human CD14 Antibody.
2. Mix well and incubate for 10 min at 2°C to 8°C.
3. Wash by adding 2 mL Isolation buffer and centrifuge for 8 min at 350 × g at 2°C to 8°C.
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 with rolling and tilting at 2°C to 8°C.
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 CD14 negative cells.
9. Repeat steps 7–8 to wash the bead-bound $CD14^+$ 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 for 10 min with rolling and tilting at 2°C to 8°C. Important to keep the cells cold.
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 human $CD14^+$ T cells. This protocol is scalable from 1×10^7 to 5×10^8 PBMC.

Step	Step description	Volumes per 5×10^7 PBMC	Volumes per 5×10^8 PBMC
	Recommended tube size	5 mL	15 mL
	Recommended magnet	DynaMag™-5	DynaMag™-15
1	Cell Volume	500 µL	5 mL
1	FlowComp™ Human CD14 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 x1 mL	2 x 10 mL
10	FlowComp™ Release Buffer	1 mL	10 mL
14*	Wash cells (Isolation Buffer)	2 mL	20 mL

* Adjust the Isolation Buffer volumes (steps 3 and 14) 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

FlowComp™ Dynabeads® are uniform, superparamagnetic polystyrene beads (2.8 µm in diameter) coated with modified streptavidin. FlowComp™ Human CD14 Antibody contains a DSB-X conjugated monoclonal mouse anti-human CD14. 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
Anti-CD14 clone Tuk4	MHCD1404
Phosphate buffered saline	14190
SYTOX® Red	S34859

REF on labels is the symbol for catalog number.

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