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Dynabeads® FlowComp™ Human CD14

Isolation directly from buffy coat and whole blood

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

Whole blood: 80 mL
Buffy coat: 160 mL

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 magnetic isolation of CD14+ monocytes directly from anti-coagulated whole blood or buffy coat. 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 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®.

Downstream Applications

Isolated cells are bead-free and may be used directly in any downstream application including flow cytometry, cell culture, functional assays or differentiation into monocyte-derived dendritic cells.

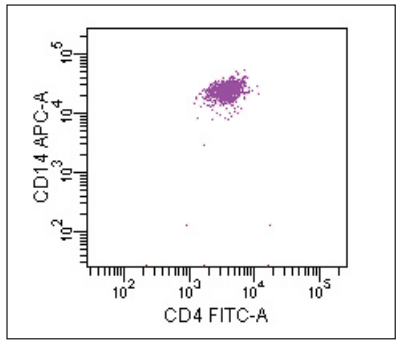

Figure 1: Purity of human CD14+ cells isolated from buffy coat using Dynabeads® FlowComp™ Human CD14.

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 antibodies. 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.
- 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.
- 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.

Protocols

In human whole blood from normal blood donors, approximately 15–20% of all leucocytes express CD14. This protocol describes magnetic capture and isolation of highly pure CD14+ cells from buffy coat and whole blood.

Wash the Beads

For whole blood volume recommendations, see Table 1. For buffy coat volume recommendations, see Table 2 .

1. Resuspend the beads in the vial (vortex >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 Buffer 1 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 Buffer 1 as the initial volume of beads.

Prepare Cells

- To secure a high recovery it is very important to keep all buffers and reagents cold (2°C to 8°C) during the entire isolation process.
- Collect whole blood sample in a collection tube containing an appropriate anticoagulant, (e.g. EDTA, heparin, ACD or citrate).
- Prepare approximately 30 mL of Isolation Buffer per test (2 mL whole blood or 4 mL buffy coat).
- *Optional:* Wash the buffy coat/whole blood once prior to use to remove soluble CD14 in the sample. This step will increase the total recovery of the monocytes. Wash by filling up your tube with Isolation Buffer and centrifuge 350 × g for 10 min at 2°C to 8°C without brakes. Remove pellet back to the original starting volume (leave approx. 1 cm above the red blood pellet).

Isolate Cells from Buffy Coat

This protocol is based on 4 mL buffy coat, but is scalable according to Table 1. When working with smaller volumes, do not use less reagents or buffers than the volumes recommended for 4 mL. When working with volumes >4 mL, scale up all volumes accordingly, as shown in Table 1.

1. Transfer 4 mL pre-cooled and preferable washed buffy coat to a tube on ice and add 25 µL FlowComp™ Human CD14 Antibody.
2. Mix well and incubate for 10 min at 2°C to 8°C.
3. Fill up the tube with Isolation Buffer and mix well, followed by centrifugation for 15 min at 350 × g at 2°C to 8°C with no brakes.
4. Aspirate the supernatant and discard the volume added in step 3 (but keep at least 1 cm above cell pellet to avoid monocyte loss).
5. Add 150 µL resuspended FlowComp™ Dynabeads® and mix well by vortexing.
6. Incubate for 15 min at 2°C to 8°C under rolling and tilting.
7. Add 8 mL Isolation Buffer, mix gently 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 CD14 negative cells. Be careful not to disturb the bead pellet on the tube wall - use a thin pipette.
9. Repeat steps 7–8 twice to wash the bead-bound CD14+ cells a total of three times. Use only half the amount of Isolation Buffer (4 mL) in the last wash. These steps are critical to obtain a high purity of isolated cells.

Release Cells from Buffy Coat

10. Resuspend in 1 mL FlowComp™ Release Buffer and pipet 3–4 times.
11. Incubate for 10 min at 2°C to 8°C 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 CD14+ 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+ cells from buffy coat. This protocol is scalable from 4–25 mL buffy coat.

Step	Step description	Volumes per 4 mL buffy coat	Volumes per 20 mL buffy coat
	Recommended tube size	15 mL	50 mL
	Recommended magnet	DynaMag™-15	DynaMag™-50
1	Buffy coat	4 mL	20 mL
1	FlowComp™ Human CD14 Antibody	25 µL	125 µL
3	Wash cells (Isolation Buffer)	~8 mL	~40 mL
5*	FlowComp™ Dynabeads®	150 µL	750 µL
7–9	Wash beads (Isolation Buffer)	2×8 mL + 1×4 mL	2×40 mL +1×20 mL
10**	FlowComp™ Release Buffer	1 mL	5 mL
14	Wash cells (Buffer 2)	2 mL	10 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.

**Transfer the sample to a smaller tube during the release to avoid cell loss on the tube wall.

Isolate Cells from Whole Blood

This protocol is based on 2 mL whole blood, but is scalable according to Table 2. When working with smaller volumes, do not use less reagents or buffers than the volumes recommended for 2 mL. When working with volumes >2 mL, scale up all volumes accordingly, as shown in Table 2.

1. Transfer 2 mL pre-cooled and preferable washed whole blood to a tube on ice and add 5 µL FlowComp™ Human CD14 Antibody.
2. Mix well and incubate for 10 min at 2°C to 8°C.
3. Fill up the tube with Isolation Buffer and mix well, followed by centrifugation for 15 min at 350 × g at 2°C to 8°C with no brakes.
4. Aspirate the supernatant and discard the volume added in step 3 (but keep at least 1 cm above cell pellet to avoid monocyte loss).
5. Add 75 µL resuspended FlowComp™ Dynabeads® and mix well by vortexing.
6. Incubate for 15 min at 2°C to 8°C under rolling and tilting.
7. Add 4 mL Isolation Buffer, mix gently 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 CD14 negative cells. Be careful not to disturb the bead pellet on the tube wall. Use a thin pipette.
9. Repeat steps 7–8 twice to wash the bead-bound CD14+ cells a total of three times. Use only half the amount of Isolation Buffer (2 mL) in the last wash. These steps are critical to obtain a high purity of isolated cells.

Release Cells from Whole Blood

10. Resuspend in 1 mL FlowComp™ Release Buffer and pipet 3–4 times.
11. Incubate for 10 min at 2°C to 8°C 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 CD14+ 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+ cells from whole blood. This protocol is scalable from 2–25 mL whole blood.

Step	Step description	Volumes per 2 mL buffy coat	Volumes per 20 mL buffy coat
	Recommended tube size	5 mL	50 mL
	Recommended magnet	DynaMag™-5	DynaMag™-50
1	Whole blood	2 mL	20 mL
1	FlowComp™ Human CD14 Antibody	5 µL	50 µL
3	Wash cells (Isolation Buffer)	~3 mL	~25 mL
5*	FlowComp™ Dynabeads®	150 µL	1.5 mL
7–9	Wash beads (Isolation Buffer)	2×4 mL + 1×2 mL	2×40 mL +1×20 mL
10**	FlowComp™ Release Buffer	1 mL	10 mL
14	Wash cells (Buffer 2)	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.

**Transfer the sample to a smaller tube during the release to avoid cell loss on the tube wall.

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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