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Dynabeads® FlowComp™ Flexi, part A

Isolation directly from MNC

Catalog no. 11061D **Store at 2˚C to 8˚C**

Rev. Date: May 2012 (Rev. 003)

Kit Contents

Cat. no. 11061D		
Part no.	**Product**	**Contents**
11060D*	Dynabeads® FlowComp™ Flexi, part A	FlowComp™ Dynabeads®, 3 mL FlowComp™ Release Buffer, 2 × 20 mL
D-20655	DSB-X Biotin Protein Labeling Kit	See the kit manual for details

*This component is not sold separately.

Kit capacity

PBMC: ~2 × 10^9

This manual describes the details for the Dynabeads® FlowComp™ Flexi, part A (Part no. 11060D) only. For details on how to use the DSB-X Biotin Protein Labeling Kit, refer to the manual available with the kit.

FlowComp™ Dynabeads® contains ~1.5 × 10^9 (~15 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™ 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 isolation of cells from a variety of samples and species, e.g. whole blood, MNCs, tissue digests and splenocytes/ lymph node cells (fig. 1). A separate protocol describes isolation of cells from MNC. This protocol describes separation of cells from whole blood or buffy coat. Label the antibody of choice using the supplied DSB-X Biotin Protein Labeling Kit. Incubate the cells with the DSB-X labeled antibody. Add FlowComp™ Dynabeads® to the labeled cells, and isolate the bead-bound cells using a magnet. Release the target cells from the Dynabeads® using the FlowComp™ Release Buffer.

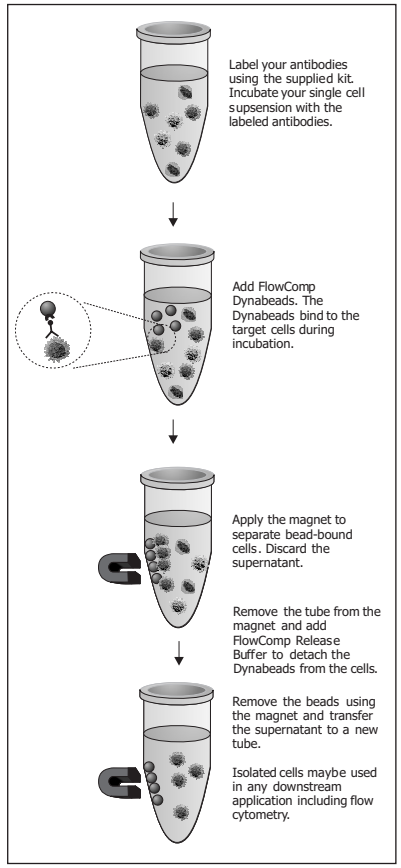

Figure 1: Method overview

Downstream Application

Isolated cells are bead-free and may be used in any downstream application including flow cytometry. Since this kit gives release under cold conditions, phagocyting cells can be isolated with this kit. A higher loss of cells is expected compared to isolation of non-phagocyting 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).
- Purified antibody without protein additives in the buffer (e.g. BSA).
- Isolation Buffer: Ca^{2+} and Mg^{2+} free PBS supplemented with 0.1% BSA and 2 mM EDTA. BSA can be replaced by human serum albumin (HSA) or 2% fetal bovine serum (FBS)/fetal calf serum (FCS). EDTA can be replaced by 0.6 % sodium citrate.

General Guidelines

- 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.
- When isolating phagocytic/adherent cells, perform the cell isolation at low temperature (2°C to 8°C or on ice).

Antibody Tips

- The choice of antibody clone is the most important factor for successful cell isolation. Not all antibodies are suitable for cell isolation with magnetic beads, although proven successful for staining.
- Very low levels of target cells may require larger amounts of antibody, longer incubation time or higher cell concentration. The concentration of antibody during coating of cells is a very important factor for good results. A general rule is to use between 40–200 µg antibody/mL PBS with 0.5% BSA (*optional:* 0.02% sodium azide). Titration of antibody towards your application is required.
- When labeling antibody with DSB-X Biotin, use purified antibody without protein additives in the buffer (e.g. BSA). Low concentrations of sodium azide (<0.09%) and Threalose (<5%) will not interfere with labeling.
- It is critical to use DSB-X biotin labeled antibodies/proteins for this kit. Standard biotinylated antibodies will not give cell release.
- For flow staining of cells after isolation use a primary fluorescent antibody that does not bind to the same epitope as your DSB-X labeled antibody. Secondary antibodies can be used.
- If spilling whole blood or buffy coat in tube cap, change cap to avoid contamination with red blood cells.

Cell Isolation Tips

- When labeling the cells with the antibody (coating), the temperature can be varied in the range of 0°C to 37°C. A temperature in the range of 2 °C to 8°C is usually preferred to reduce biological activity in the cell (e.g. enzymatic cleavage or internalization of receptors) while keeping coating time as short as possible. The time can be optimized in the range of 5–30 min. 10 min is usually sufficient. Coating on ice (compared to 2°C to 8°C) will require longer coating time (e.g. 20 min).
- When adding the beads to the labeled cells the isolation time can be optimized in the range of 5–30 min. 10–20 min is usually sufficient for optimal recovery. Use the same temperature guidelines as for the coating process. For isolation of phagocytic cells (i.e. monocytes or macrophages), keep the temperature low to avoid phagocytic activity.
- If the target cell concentration exceeds 50% of the total cells, reduce the cell concentration accordingly. Alternatively, increase the amount of beads above 75 µL/mL of cell sample. For isolation of rare cells (e.g. stem cells from BM or leukophoresis) the cell concentration should be increased up to 1 × 10^8 cells/mL.
- The release time can be optimized in the range of 2–20 min. 2–10 min is usually sufficient. Another release step can be added if necessary. One release step is usually sufficient.

- Increase the number of washing steps if necessary to increase the purity. This could affect the cell recovery.
- The total time used for cell isolation and release can influence on the recovery. A very long procedure decreases the recovery, thus it is not recommended to incubate overnight or exceed the recommended incubation times.

Protocol

This protocol describes isolation of cells from 5×10^7 MNC using Dynabeads® FlowComp™ Flexi. When working with fewer cells than 5×10^7, use the same volumes as for 5×10^7. When working with higher cell numbers, scale up all reagents and volumes accordingly, as shown in Table 1.

Wash Dynabeads®

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).

Prepare Sample

- Prepare a MNC 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.
- Titrate the antibody to optimize the amount for your target cells. Use 1–10 µg antibody per 1×10^7 target cells. A volume of 25 µL DSB-X labeled antibody is needed per 5×10^7 cells. Adjust the concentration of the labeled antibody accordingly using PBS with 0.5% BSA (*optional:* 0.02% sodium azide).

Isolate Cells

1. Add 25 µL DSB-X biotinylated antibody to 500 µL cells (5×10^7 MNC) and vortex for 2–3 sec.
2. Incubate 10 min at 2°C to 8°C.
3. Add 2 mL cold Isolation Buffer to wash the cells and centrifuge at 350 × g for 10 min at 2°C to 8°C without brakes.
4. Remove and discard the supernatant.
5. Add 1 mL cold Isolation Buffer to the cell pellet and resuspend by pipetting.
6. Add 75 µL FlowComp Dynabeads® and vortex for 2–3 sec. Optimize the bead amount for each application.
7. Incubate 15 min with rolling and tilting at 2°C to 8°C.
8. Add 1 mL Isolation Buffer, pipet 2–3 times (or vortex 2–3 sec) and place the tube in a magnet for 2 min.
9. While the tube is still in the magnet, carefully remove and discard the supernatant containing the non-target cells. Be careful not to disturb the bead-pellet on the tube wall. Use a narrow pipette.
10. Repeat step 8–9 twice to a total of three washes.
    Optional: Transfer the sample to a smaller tube to minimize loss of cells on the tube wall during release.
11. Resuspend the cells in 1 mL Release Buffer. Be careful to resuspend all beads trapped on the tube wall.
12. Incubate for 10 min with rolling and tilting at 2°C to 8°C.
13. Pipet 10 times to efficiently release the cells and place in a magnet for 1 min. Avoid air bubbles.
14. Carefully transfer the supernatant containing the bead-free cells to a new tube. Be careful not to disturb the bead pellet on the tube wall. Use a narrow pipette.
15. Place the cells in the magnet again for 1 min to remove residual beads, and transfer the supernatant containing the bead-free cells to a new tube.

The isolated cells can be used directly for flow staining. For cell culture; centrifuge and resuspend the cells in a suitable cell culture media.

Table 1: Volumes for isolation of cells from MNC.

Step	Step description	Volumes per 5×10^7 MNC	Volumes per 5×10^8 MNC
	Recommended tube size	5 mL	50 mL
	Recommended magnet	DynaMag™-5	DynaMag™-50
1	DSB-X biotinylated antibody	25 µL	250 µL
1	MNC	500 µL	5 mL
3*	Wash cells (Isolation Buffer)	~2 mL	20 mL
5	Resuspend cells (Isolation Buffer)	1 mL	10 mL
6**	FlowComp™ Dynabeads®	75 µL	750 µL
8–10*	Wash beads (Isolation Buffer)	3 × ~1 mL	3 × ~10 mL
11	FlowComp™ Release Buffer	1 mL	10 mL

* Adjust the Isolation Buffer volumes to fit to the tube you are using.

** When incubating, tilt and rotate 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 polymer coated beads (2.8 µm diameter) coated with recombinant streptavidin. The FlowComp™ Release Buffer contains modified biotin supplied in PBS with 0.1% BSA and 2 mM EDTA.

Related Products

Product	Cat. no.
DynaMag™-5	12303D
DynaMag™-15	12301D
DynaMag™-50	12302D
HulaMixer® Sample Mixer	15920D
DSB-X Biotin Protein Labeling Kit	D20655

REF on labels is the symbol for catalog number.

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