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# Dynabeads® FlowComp™ Flexi, part A

## Isolation directly from whole blood/buffy coat

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

**Rev. Date:** May 2012 (Rev. 001)

## Kit Contents

| Cat. no. 11061D | | |
|---|---|---|
| **Part no.** | **Product** | **Contents** |
| 11060D* | Dynabeads® FlowComp™ Flexi, part A | FlowComp™ Dynabeads®, 3 mL<br>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

Whole blood/buffy coat: ~80 mL

**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, mononuclear cells (MNC), tissue digests and splenocytes/lymph node cells (fig 1.). This protocol describes separation of cells from MNC.
A separate protocol describes isolation 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.

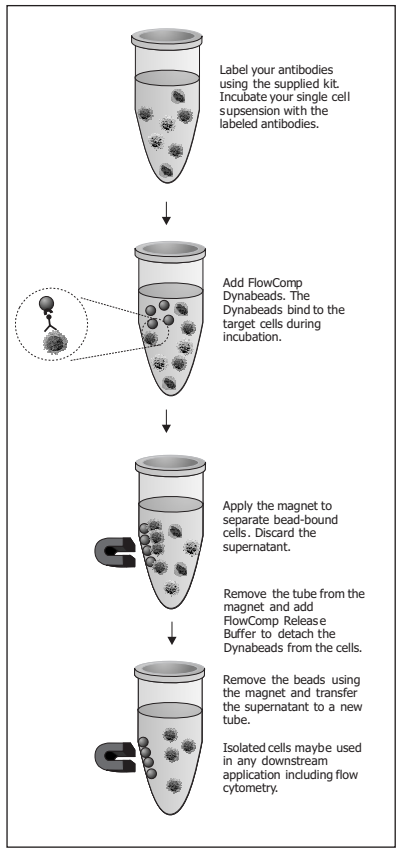


Figure 1: Method overview

### 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.

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## Protocol

This protocol is intended for isolation of bead-free cells directly from buffy coat or whole blood. One test is defined as 2 mL of buffy coat or whole blood. The protocol is directly scalable; when working with volumes <2 mL, use the same volumes as described for 2 mL. When working with >2 mL, scale up all reagents and total 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

- Visit www.lifetechnologies.com/samplepreparation for recommended sample preparation procedures.
- It is recommended to wash whole blood/buffy coat prior to cell isolation to remove any soluble proteins that could interfere with the binding of the labeled antibody to the target cells. Add Isolation Buffer to the tube containing your sample and centrifuge at 350 × g for 10 min without brakes. Remove supernatant, but leave the liquid approximately 1 cm above the red blood cell pellet.
- Titrate the antibody to optimize the amount for your target cells. Use 1–10 µg antibody per $1 \times 10^7$ target cells. A volume of 25 µL DSB-X labeled antibody is needed per $5 \times 10^7$ 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 2 mL buffy coat and vortex for 2–3 sec.
2. Incubate 10 min at 2°C to 8°C.
3. Fill tube with Isolation Buffer and centrifuge at 350 × g for 10 min at 2°C to 8°C without brakes.
4. Remove supernatant, leaving approximately 1 cm above the red blood cell pellet.
5. Add 75 µL FlowComp™ Dynabeads® and vortex for 2–3 sec. Optimize the bead amount for each application.
6. Incubate 15 min with rolling and tilting at 2°C to 8°C.
7. Add 4 mL Isolation Buffer and pipet slowly twice before placing the tube in a magnet for 2–3 min.
8. 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.
9. Repeat steps 7–8 twice to a total of three washes. In the final wash, reduce the Isolation Buffer to 2 mL.
   *Optional:* Transfer the sample to a smaller tube to minimize loss of cells on the tube wall during release.
10. Resuspend the cells in 1 mL Release Buffer. Be careful to resuspend all beads trapped on the tube wall.
11. Incubate for 10 min with rolling and tilting at 2°C to 8°C.
12. Pipet 10 times to efficiently release the cells and place in a magnet for 1 min. Avoid air bubbles.
13. 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.
14. 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 whole blood/buffy coat.

| Step | Step description | Volumes small scale (1X) | Volumes large scale (10X) |
|---|---|---|---|
| | Recommended tube size | 5 mL | 50 mL |
| | Recommended magnet | DynaMag™-5 | DynaMag™-50 |
| 1 | DSB-X biotinylated antibody | 25 µL | 250 µL |
| 1 | Whole blood/buffy coat | 2 mL | 20 mL |
| 3* | Wash cells (Isolation Buffer) | ~2 mL | 20 mL |
| 5** | FlowComp™ Dynabeads® | 75 µL | 750 µL |
| 7–9* | Wash beads (Isolation Buffer) | ~4 mL + 4 mL + 2 mL | ~40 mL + 40 mL + 20 mL |
| 10 | 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

Dynabeads® FlowComp™ 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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