# EasySep™ Direct Human B Cell Isolation Kit

**For processing 100 mL whole blood**

Catalog #19674
Catalog #19674RF RoboSep™

Negative Selection

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## Description

Isolate highly purified B cells directly from human whole blood by immunomagnetic negative selection.

The benefits of this kit include:

- > 99.9% RBC depletion without the need for density gradient centrifugation, sedimentation, or lysis
- Up to 95% purity of isolated cells
- Fast, easy-to-use, and column-free
- Isolated cells are untouched

This kit targets non-B cells for removal with antibodies recognizing specific cell surface markers. Unwanted cells are labeled with antibodies and EasySep™ Direct RapidSpheres™ and separated using an EasySep™ magnet. Desired cells are simply collected into a new tube and are immediately available for downstream applications, such as flow cytometry, culture, or DNA/RNA extraction.

## Component Descriptions

| COMPONENT NAME | COMPONENT # | QUANTITY | STORAGE | SHELF LIFE | FORMAT |
|---|---|---|---|---|---|
| EasySep™ Direct Human B Cell Isolation Cocktail | 19674C | 2 x 2.5 mL | Store at 2 - 8°C. Do not freeze. | Stable until expiry date (EXP) on label. | A combination of monoclonal antibodies in PBS. |
| EasySep™ Direct RapidSpheres™ 50300 | 50300 | 4 x 2.5 mL | Store at 2 - 8°C. Do not freeze. | Stable until expiry date (EXP) on label. | A suspension of magnetic particles and monoclonal antibodies in PBS. |

PBS - phosphate-buffered saline

Components may be shipped at room temperature (15 - 25°C) and should be refrigerated upon receipt.

Precipitate may be observed in the cocktail vial but will not affect performance.

## Sample Preparation

PERIPHERAL BLOOD

For optimal RBC depletion, collect blood using heparin or acid-citrate-dextrose (ACD) as an anticoagulant. The use of K2EDTA or K3EDTA as an anticoagulant is not recommended.

For best recovery, use unprocessed human whole blood. Recovery of the desired isolated cells decreases with samples that are older than 24 hours.

The volume of blood that can be processed depends on the EasySep™ magnet used for the isolation procedure. Blood samples must be placed in the required tube to properly fit into the appropriate EasySep™ magnet (see Tables 1 and 2).

BUFFY COAT (OPTIONAL - FOR USE WITH ROBOSEP™)

1. Add an equal volume of recommended medium to whole blood.
2. Centrifuge at 800 x g for 10 minutes at room temperature (15 - 25°C) with the brake off.
3. Remove the concentrated leukocyte band (this is the buffy coat), plus a small portion of the plasma and concentrated red blood cells (RBCs). The target is to concentrate the leukocytes approximately 5-fold while maintaining the same hematocrit (e.g. collect 2 mL of buffy coat when starting with 10 mL of whole blood).
4. Transfer buffy coat to the required tube (see Table 3).

## Recommended Medium

PBS (Catalog #37350) that is free of Ca++ and Mg++.

## Directions for Use – Manual EasySep™ Protocols

See page 1 for Sample Preparation and Recommended Medium. Refer to Tables 1 and 2 for detailed instructions regarding the EasySep™ procedure for each magnet.

**Table 1. EasySep™ Direct Human B Cell Isolation Kit Protocol**

| | | EASYSEP™ MAGNETS | |
|---|---|---|---|
| STEP | INSTRUCTIONS |  **EasySep™ (Catalog #18000)** | **"The Big Easy" (Catalog #18001)**  |
| 1 | Collect sample within the volume range. | 0.5 - 1.5 mL | 1.5 - 7.0 mL |
| | Add whole blood sample to required tube. | 5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007) | 14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008) |
| 2 | Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed. | 30 seconds | 30 seconds |
| 3 | Add Isolation Cocktail to sample. NOTE: Do not vortex cocktail. | 50 µL/mL of sample | 50 µL/mL of sample |
| 4 | Add RapidSpheres™ to sample. | 50 µL/mL of sample | 50 µL/mL of sample |
| | Mix and incubate. | RT for 5 minutes | RT for 5 minutes |
| 5 | Add recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times. | Top up to 2.5 mL | • Top up to double the volume for samples ≤ 5 mL<br>• Top up to 10 mL for samples > 5 mL |
| 6 | Place the tube (without lid) into the magnet and incubate. | RT for 5 minutes | RT for 5 minutes |
| 7 | Pick up the magnet, and in one continuous motion invert the magnet and tube, pouring the enriched cell suspension* into a new tube. | Use a new 5 mL tube | Use a new 14 mL tube |
| 8 | Add RapidSpheres™ to the new tube containing the enriched cells. | Use same volume as in step 4 | Use same volume as in step 4 |
| | Mix and incubate. | RT for 5 minutes | RT for 5 minutes |
| 9 | Remove the tube from the magnet; place the tube from step 8 (without lid) into the magnet and incubate for a second separation. | RT for 5 minutes | RT for 5 minutes |
| 10 | Pick up the magnet, and in one continuous motion invert the magnet and tube,** pouring the enriched cell suspension into a new tube. | Isolated cells are ready for use | Use a new 14 mL tube |
| 11 | Remove the tube from the magnet; place the new tube from step 10 (without lid) into the magnet and incubate for a third separation. | --- | RT for 5 minutes |
| 12 | Pick up the magnet, and in one continuous motion invert the magnet and tube,** pouring the enriched cell suspension into a new tube. | --- | Isolated cells are ready for use |

RT - room temperature (15 - 25°C)
* Following the first magnetic separation, the collected cells may contain a significant amount of RBCs and may look similar to the original unprocessed human whole blood sample.
** To minimize RBC contamination in the isolated cells, pour off the sample along a clean area of the tube (i.e. the opposite side to where the sample was poured in).

**Table 2. EasySep™ Direct Human B Cell Isolation Kit Protocol**

| | | EASYSEP™ MAGNETS | | |
|---|---|---|---|---|
| | | EasyEights™ (Catalog #18103) | | |
| STEP | INSTRUCTIONS | 5 mL tube | 14 mL tube | Easy 50 (Catalog #18002) |
| 1 | Collect sample within the volume range. | 0.5 - 1.5 mL | 1.5 - 7.0 mL | 7 - 30 mL |
| | Add whole blood sample to required tube. | 5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007) | 14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008) | 50 mL (30 x 115 mm) conical tube (e.g. Catalog #38010) |
| 2 | Vortex RapidSpheres™.<br>NOTE: Particles should appear evenly dispersed. | 30 seconds | 30 seconds | 30 seconds |
| 3 | Add Isolation Cocktail to sample.<br>NOTE: Do not vortex cocktail. | 50 µL/mL of sample | 50 µL/mL of sample | 50 µL/mL of sample |
| 4 | Add RapidSpheres™ to sample. | 50 µL/mL of sample | 50 µL/mL of sample | 50 µL/mL of sample |
| | Mix and incubate. | RT for 5 minutes | RT for 5 minutes | RT for 5 minutes |
| 5 | Add recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times. | Top up to 2.5 mL | • Top up to double the volume for samples ≤ 5 mL<br>• Top up to 10 mL for samples > 5 mL | • Top up to double the volume for samples ≤ 25 mL<br>• Top up to 50 mL for samples > 25 mL |
| 6 | Place the tube (without lid) into the magnet and incubate. | RT for 5 minutes | RT for 5 minutes | RT for 10 minutes |
| 7 | Carefully pipette*** (do not pour) the enriched cell suspension into a new tube.<br>NOTE: Collect the entire clear fraction from top to bottom. For optimal recovery, also collect a small volume of RBCs (up to 10% of the starting sample volume). | Use a new 5 mL tube | Use a new 14 mL tube | Use a new 50 mL tube |
| 8 | Add RapidSpheres™ to the new tube containing the enriched cells. | 25 µL/mL of original sample volume | 25 µL/mL of original sample volume | Use same volume as in step 4 |
| | Mix and incubate. | RT for 5 minutes | RT for 5 minutes | RT for 5 minutes |
| 9 | Remove the tube from the magnet; place the tube from step 8 (without lid) into the magnet and incubate for a second separation. | RT for 5 minutes | RT for 5 minutes | RT for 5 minutes |
| 10 | Carefully pipette*** (do not pour) the enriched cell suspension into a new tube.<br>NOTE: Collect only the clear fraction. | Use a new 5 mL tube | Use a new 14 mL tube | Use a new 50 mL tube |
| 11 | Add RapidSpheres™ to the new tube containing the enriched cells. | 25 µL/mL of original sample volume | 25 µL/mL of original sample volume | --- |
| | Mix and incubate. | RT for 5 minutes | RT for 5 minutes | --- |
| 12 | Remove the tube from the magnet; place the new tube (without lid) containing the enriched cells into the magnet and incubate for a third separation. | RT for 5 minutes | RT for 5 minutes | RT for 5 minutes |
| 13 | Carefully pipette*** (do not pour) the enriched cell suspension into a new tube.<br>NOTE: Collect only the clear fraction. | Isolated cells are ready for use | Isolated cells are ready for use | Isolated cells are ready for use |

RT - room temperature (15 - 25°C)
*** Collect the entire enriched cell suspension, all at once, into a single pipette (e.g. for EasyEights™ 5 mL tube, use a 2 mL serological pipette [Catalog #38002]; for EasyEights™ 14 mL tube, use a 10 mL serological pipette [Catalog #38004]).


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## Directions for Use – Fully Automated RoboSep™ Protocol

See page 1 for Sample Preparation and Recommended Medium. Refer to Table 3 for detailed instructions regarding the RoboSep™ procedure.
NOTE: If using RoboSep™-S, ensure the software is at least v.1.2.0.2 and RoboSep™ Direct-compatible carousel is installed. Contact us at techsupport@stemcell.com for more information.

**Table 3. RoboSep™ Direct Human B Cell Isolation Kit Protocol**

| STEP | INSTRUCTIONS | RoboSep™ (Catalog #21000)  |
|---|---|---|
| 1 | Prepare sample within the volume range. | **For blood:** 1 - 6 mL<br>**For buffy coat:** 2 - 6 mL |
| | Add sample to required tube. | 14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008) |
| 2 | Select protocol. | • EasySep Direct Human B Cell Isolation 19674 - whole blood<br>• EasySep Direct Human B Cell Isolation 19674 - buffy coat§ |
| 3 | Vortex RapidSpheres™.<br>NOTE: Particles should appear evenly dispersed. | 30 seconds |
| 4 | Load the carousel. | Follow on-screen prompts<br>NOTE: This protocol requires loading **two** vials of EasySep™ Direct RapidSpheres™ 50300 onto the carousel for a single run; one in the ▲ (triangle) slot and one in the ● (circle) slot. |
| | Start the protocol. | Press the green "Run" button |
| 5 | Unload the carousel when the run is complete. | Isolated cells are ready for use |

§ This protocol uses two times the EasySep™ reagents

## Notes and Tips

REMOVAL OF RESIDUAL RBCs IN ISOLATED CELLS

Typically, further RBC depletion is not required following cell isolation. If residual RBCs are visible in the isolated cell pellet following centrifugation after the end of the protocol, resuspend in a small volume (0.2 - 2.5 mL) of recommended medium or desired culture medium and place in a smaller EasySep™ magnet for an additional 5-minute separation. Collect the supernatant; the isolated cells are ready for use in downstream applications. Residual RBCs may also be lysed using Ammonium Chloride Solution (Catalog #07800).

ASSESSING PURITY

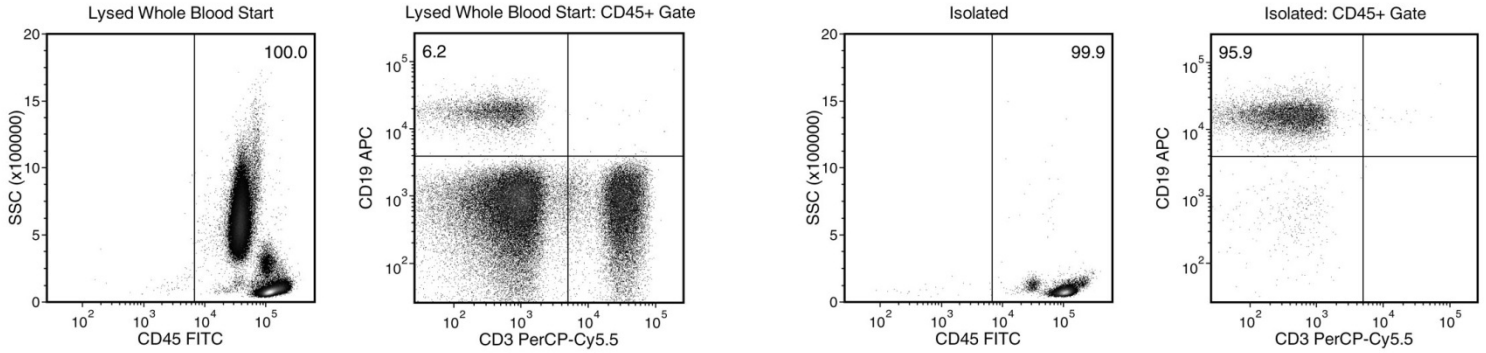
For purity assessment of B cells by flow cytometry, use the following fluorochrome-conjugated antibody clones:

- Anti-Human CD19 Antibody, Clone HIB19 (Catalog #60005), or
- Anti-Human CD20 Antibody, Clone 2H7 (Catalog #60008), and
- Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018)

NOTE: It is recommended to assess purity on CD45-positive cells to exclude debris, platelets, and RBCs. Include a viability dye if necessary (e.g. Propidium Iodide [Catalog #75002]; 7-AAD [7- Aminoactinomycin D; Catalog #75001]).

## Data

Starting with human whole blood from normal healthy donors, the typical B cell (CD3-CD19+) content of the non-lysed final isolated fraction is 95.3 ± 2.7% (gated on CD45) or 88.5 ± 11.5% (not gated on CD45).




In the above example, the B cell (CD3-CD19+) content of the lysed whole blood start sample and the non-lysed final isolated fraction is 6.2% and 95.9% (gated on CD45), respectively, or 6.2% and 95.8% (not gated on CD45), respectively. The starting frequency of B cells in the non-lysed whole blood start sample above is 0.011% (data not shown).

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