# EasySep™ Non-Human Primate B Cell Isolation Kit

**For processing 1 x 10^9 cells**

Catalog #100-0345
Catalog #100-0347 RoboSep™

Negative Selection

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## Description

Isolate untouched and highly purified B cells from fresh or previously frozen non-human primate peripheral blood mononuclear cells (PBMCs) in as little as 20 minutes by immunomagnetic negative selection.

- Fast, easy-to-use and column-free
- Up to 95% purity
- Untouched, viable cells

This kit targets non-B cells for removal with antibodies recognizing specific cell surface markers. Unwanted cells are labeled with antibodies and magnetic particles, and separated without columns using an EasySep™ magnet. Desired cells are simply poured off into a new tube. Isolated cells 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™ Non-Human Primate B Cell Isolation Cocktail | 300-0150 | 1 x 1 mL | Store at 2 - 8°C. Do not freeze. | Stable until expiry date (EXP) on label. | A combination of monoclonal antibodies in PBS and 0.09% sodium azide. |
| EasySep™ Dextran RapidSpheres™ 50102 | 50102 | 2 x 1 mL | Store at 2 - 8°C. Do not freeze. | Stable until expiry date (EXP) on label. | A suspension of magnetic particles in PBS. |
| EasySep™ Isolation Cocktail Enhancer | 17900 | 1 x 1 mL | Store at 2 - 8°C. Do not freeze. | Stable until expiry date (EXP) on label. | A solution that enhances the performance of the isolation cocktail. |

PBS - phosphate-buffered saline

Components may be shipped at room temperature (15 - 25°C) but should be stored as indicated above.

## Sample Preparation

This kit has been verified for use with rhesus and cynomolgus macaques.

PERIPHERAL BLOOD

For peripheral whole blood from rhesus macaques, prepare a PBMC suspension by centrifugation over a density gradient medium (e.g. Lymphoprep™, Catalog #07801). For peripheral whole blood from cynomolgus macaques, dilute the density gradient medium to 90% using D-PBS (Without Ca++ and Mg++; Catalog #37350).

NOTE: For higher recovery, 15 mL conical tubes (e.g. Catalog #38009) are recommended for density gradient centrifugation, particularly for smaller volumes of peripheral blood.

For samples > 24 hours old, it may be necessary to lyse the red blood cells (RBCs) using Ammonium Chloride Solution (Catalog #07800) prior to cell isolation.

If using previously frozen PBMCs, incubate the cells with DNase I Solution (Catalog #07900) at a concentration of 100 µg/mL at room temperature (15 - 25°C) for at least 15 minutes prior to labeling and separation. Filter aggregated suspensions through a 37 µm cell strainer (e.g. Catalog #27250) for optimal results.

After preparation, resuspend cells at 5 x 10^7 cells/mL in recommended medium.

## Recommended Medium

EasySep™ Buffer (Catalog #20144), RoboSep™ Buffer (Catalog #20104), or PBS containing 2% FBS and 1 mM EDTA. Medium should be free of Ca++ and Mg++.

## Directions for Use – Manual EasySep™ Protocols

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

**Table 1. EasySep™ Non-Human Primate B Cell Isolation Kit Protocol**

| | | EASYSEP™ MAGNETS | |
|---|---|---|---|
| STEP | INSTRUCTIONS |  **EasySep™ (Catalog #18000)** | **"The Big Easy" (Catalog #18001)**  |
| 1 | **Prepare sample at the indicated cell concentration within the volume range.** | 5 x 10^7 cells/mL<br>0.5 - 2 mL | 5 x 10^7 cells/mL<br>0.5 - 6 mL |
| | **Add sample to required tube.** | 5 mL (12 x 75 mm) polystyrene round-bottom tube<br>(e.g. Catalog #38007) | 14 mL (17 x 95 mm) polystyrene round-bottom tube<br>(e.g. Catalog #38008) |
| 2 | **Add Cocktail Enhancer to sample.** | 50 µL/mL of sample | 50 µL/mL of sample |
| | **Mix and incubate.** | RT for 1 minute | RT for 1 minute |
| 3 | **Add Isolation Cocktail to sample.** | 50 µL/mL of sample | 50 µL/mL of sample |
| | **Mix and incubate.** | RT for 10 minutes | RT for 10 minutes |
| 4 | **Vortex RapidSpheres™.**<br>**NOTE: Particles should appear evenly dispersed.** | 30 seconds | 30 seconds |
| 5 | **Add RapidSpheres™ to sample and mix.** | 75 µL/mL of sample<br>No incubation, IMMEDIATELY proceed to next step | 75 µL/mL of sample<br>No incubation, IMMEDIATELY proceed to next step |
| 6 | **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 5 mL for samples < 2 mL<br>• Top up to 10 mL for samples ≥ 2 mL |
| | **Place the tube (without lid) into the magnet and incubate.** | RT for 3 minutes | RT for 3 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 | **Remove the tube from the magnet and place the new tube from step 7 (without lid) into the magnet and incubate for a second separation.**** | RT for 3 minutes** | RT for 3 minutes** |
| 9 | **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<br>Isolated cells are ready for use | Use a new 14 mL tube<br>Isolated cells are ready for use |

RT - room temperature (15 - 25°C)
* Leave the magnet and tube inverted for 2 - 3 seconds, then return upright. Do not shake or blot off any drops that may remain hanging from the mouth of the tube.
** Incubation time may be reduced to 1 minute for some samples. See Notes and Tips.

## Directions for Use – Fully Automated RoboSep™ Protocol

See page 1 for Sample Preparation and Recommended Medium. Refer to Table 2 for detailed instructions regarding the RoboSep™ procedure.

**Table 2. RoboSep™ Non-Human Primate B Cell Isolation Kit Protocol**

| STEP | INSTRUCTIONS | RoboSep™ (Catalog #20000 and #21000)  |
|---|---|---|
| 1 | Prepare sample at the indicated cell concentration within the volume range. | 5 x 10^7 cells/mL<br>0.5 - 6 mL |
| | Add sample to required tube. | 14 mL (17 x 95 mm) polystyrene round-bottom tube<br>(e.g. Catalog #38008) |
| 2 | Select protocol. | Non-Human Primate B Cell Isolation |
| 3 | Vortex RapidSpheres™.<br>NOTE: Particles should appear evenly dispersed. | 30 seconds |
| 4 | Load the carousel. | Follow on-screen prompts |
| | Start the protocol. | Press the green “Run” button |
| 5 | Unload the carousel when the run is complete. | Isolated cells are ready for use |

## Notes and Tips

ASSESSING PURITY

NOTE: Due to the presence of residual RBCs, use of a CD45 antibody is strongly recommended.
NOTE: Use of a cell viability dye is strongly recommended.

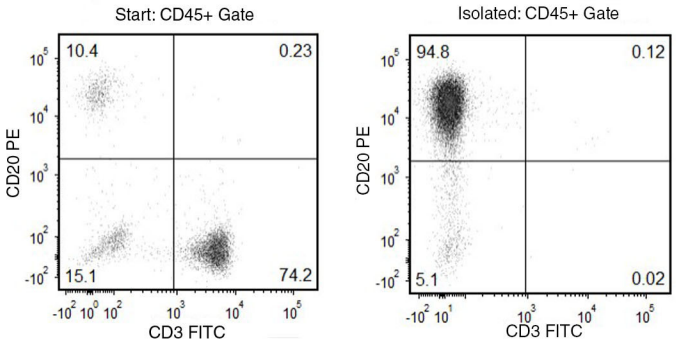
For purity assessment of non-human primate B cells by flow cytometry, use the following fluorochrome-conjugated antibodies:

- Anti-Human CD20 Antibody, Clone 2H7 (Catalog #60008)
- Anti-human CD3 antibody, clone SP34.2

OPTIMIZING RECOVERY

For increased recovery of desired cells, the second separation in Table 1, step 8 of the protocol can be reduced to 1 minute. Please note that this will likely reduce purity.

## Data




Starting with rhesus PBMCs, the B cell content (CD20+) of the isolated fraction is typically 91.4 ± 5.2% (mean ± SD using the purple EasySep™ Magnet). In the above example, the purities of the start and final isolated fractions are 10.4% and 94.8%, respectively.