# EasySep™ Human IgG+ Memory B Cell Isolation Kit

Positive Selection

Catalog #17868

For processing 2 x 10^9 cells

STEMCELL™ TECHNOLOGIES
Scientists Helping Scientists™ | WWW.STEMCELL.COM
TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713
INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM
FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

Document #DX21963 | Version 1_0_0

## Description

Isolate IgG+ memory B cells from fresh or previously frozen human peripheral blood mononuclear cells (PBMCs) or leukapheresis samples by immunomagnetic positive and negative selection.

- Highly purified IgG+ memory B cells isolated in less than 40 minutes
- No-wash removal of EasySep™ Releasable RapidSpheres™
- Optional isolation of untouched naïve B cells from the same sample

First, CD27+ cells are isolated by column-free immunomagnetic positive selection using antibody complexes and EasySep™ Releasable RapidSpheres™. Then, bound magnetic particles are removed from the EasySep™-isolated CD27+ cells, and unwanted non-B cells, as well as IgM+, IgD+, and IgA+ cells, are targeted for depletion using antibody complexes and EasySep™ Dextran RapidSpheres™. The final isolated fraction contains IgG+CD27+ memory B cells that are immediately ready for downstream applications. An optional protocol allows for the isolation of naïve B cells in parallel for use in functional studies. Following cell isolation with this EasySep™ Release kit, antibody complexes remain bound to the cell surface and may interact with Brilliant Violet™ antibody conjugates, polyethylene glycol-modified proteins, or other chemically related ligands.

## Component Descriptions

| COMPONENT NAME | COMPONENT # | QUANTITY | STORAGE | SHELF LIFE | FORMAT |
|---|---|---|---|---|---|
| EasySep™ Human CD27 Positive Selection Cocktail | 17864C | 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 0.1% BSA. Includes an Fc receptor blocking antibody. |
| EasySep™ Human Memory B Cell Pre-Enrichment Cocktail | 17454C | 2 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. |
| EasySep™ Human IgM/IgD/IgA Depletion Cocktail | 17860C | 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 0.1% BSA. |
| EasySep™ Releasable RapidSpheres™ 50201 | 50201 | 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 water. |
| EasySep™ Dextran RapidSpheres™ 50103 | 50103 | 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 water. |
| EasySep™ Release Buffer | 20145 | 2 x 1 mL | Store at 2 - 8°C. Do not freeze. | Stable until expiry date (EXP) on label. | A buffer for release of Releasable RapidSpheres™ from cells following positive selection. |

BSA - bovine serum albumin; PBS - phosphate-buffered saline

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

## Sample Preparation

For available fresh and frozen samples, see www.stemcell.com/primarycells.

PERIPHERAL BLOOD

Prepare a PBMC suspension from whole blood by centrifugation over a density gradient medium (e.g. Lymphoprep™, Catalog #07801). For more rapid PBMC preparation, use the SepMate™ RUO (Catalog #86450/86415) or SepMate™ IVD* (Catalog #85450/85415) cell isolation tube.

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 (Catalog #27250) for optimal results.

After preparation, resuspend cells at 1 x 10^8 cells/mL in recommended medium.

* SepMate™ IVD is only available in select regions where it is registered as an In Vitro Diagnostic (IVD) device for the isolation of mononuclear cells (MNCs) from whole blood or bone marrow by density gradient centrifugation. In all other regions SepMate™ is available for research use only (RUO).

LEUKAPHERESIS

If working with large volumes (> 150 mL), concentrate leukapheresis sample first by centrifuging at 500 x g for 10 minutes. Remove the supernatant and resuspend the cells in 1/10th of the original leukapheresis volume with recommended medium (e.g. for 300 mL of cells, resuspend in 30 mL of recommended medium). For small volumes (≤ 150 mL), add Ammonium Chloride Solution (Catalog #07800) directly to the leukapheresis sample.

1. Add an equal volume of Ammonium Chloride Solution to the leukapheresis sample.
2. Incubate on ice for 15 minutes.
3. Centrifuge at 500 x g for 10 minutes at room temperature (15 - 25°C). Remove the supernatant.
4. Wash the cells by topping up the tube with recommended medium. Centrifuge the cells at 150 x g for 10 minutes at room temperature with the brake off. Carefully remove the supernatant.
5. Repeat step 4 one or more times until most of the platelets have been removed (indicated by a clear supernatant).
6. Resuspend the cells at 1 x 10^8 cells/mL in recommended medium.

## Recommended Medium

EasySep™ Buffer (Catalog #20144) or PBS containing 2% fetal bovine serum (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 Tables 1 and 2 for detailed instructions regarding the EasySep™ procedure for each magnet.

**Table 1. EasySep™ Human IgG+ Memory B Cell Isolation Kit Protocol**

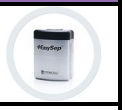
| | | EASYSEP™ MAGNETS | |
|---|---|---|---|
| STEP | INSTRUCTIONS |  **EasySep™ (Catalog #18000)** | **"The Big Easy" (Catalog #18001)**  |
| 1 | Prepare sample at the indicated cell concentration within the volume range. | 1 x 10^8 cells/mL<br>0.25 - 2 mL | 1 x 10^8 cells/mL<br>0.5 - 6 mL |
| | Add 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 CD27 Positive Selection Cocktail to sample. | 50 µL/mL of sample | 50 µL/mL of sample |
| | Mix and incubate. | RT for 3 minutes | RT for 3 minutes |
| 3 | Vortex Releasable RapidSpheres™.<br>NOTE: Particles should appear evenly dispersed. | 30 seconds | 30 seconds |
| | Add Releasable RapidSpheres™ to sample and mix. | 100 µL/mL of sample | 100 µL/mL of sample |
| 4 | Add Memory B Cell Pre-Enrichment Cocktail to sample. | 100 µL/mL of sample | 100 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 1.25 mL for samples < 1 mL<br>• Top up to 2.5 mL for samples ≥ 1 mL | • Top up to 2.5 mL for samples < 2 mL<br>• Top up to 5 mL for samples ≥ 2 - 4 mL<br>• Top up to 10 mL for samples > 4 mL |
| | Place the tube (without lid) into the magnet and incubate. | RT for 5 minutes | RT for 5 minutes |
| 6 | Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring the supernatant into a new tube. | Use a new 5 mL tube<br>Set aside supernatant for isolating naïve B cells (Table 3) if desired | Use a new 14 mL tube<br>Set aside supernatant for isolating naïve B cells (Table 3) if desired |
| 7 | Remove the tube from the magnet and 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 1.25 mL for samples < 1 mL<br>• Top up to 2.5 mL for samples ≥ 1 mL | • Top up to 2.5 mL for samples < 2 mL<br>• Top up to 5 mL for samples ≥ 2 - 4 mL<br>• Top up to 10 mL for samples > 4 mL |
| | Place the tube (without lid) into the magnet and incubate. | RT for 3 minutes | RT for 3 minutes |
| 8 | Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring off the supernatant. | Discard supernatant | Discard supernatant |
| 9 | Repeat steps as indicated. | Steps 7 and 8, two more times<br>(total of 1 x 5-minute and 3 x 3-minute separations) | Steps 7 and 8, two more times<br>(total of 1 x 5-minute and 3 x 3-minute separations) |
| Continue to step 10, next page | | Continue to step 10, next page | Continue to step 10, next page |

| | | EASYSEP™ MAGNETS | |
|---|---|---|---|
| STEP | INSTRUCTIONS (CONTINUED) |  **EasySep™ (Catalog #18000)** | **"The Big Easy" (Catalog #18001)**  |
| 10 | Remove the tube from the magnet and add recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times. Be sure to collect cells off the sides of the tube. | Same volume as the original starting sample volume (i.e. same volume used in step 1) | Same volume as the original starting sample volume (i.e. same volume used in step 1) |
| 11 | Add Release Buffer to sample. | 100 µL/mL of sample | 100 µL/mL of sample |
| | Mix and incubate. | RT for 3 minutes | RT for 3 minutes |
| 12 | Add IgM/IgD/IgA Depletion Cocktail to sample. | 100 µL/mL of sample | 100 µL/mL of sample |
| | Mix and incubate. | RT for 5 minutes | RT for 5 minutes |
| 13 | Vortex Dextran RapidSpheres™. NOTE: Particles should appear evenly dispersed. | 30 seconds | 30 seconds |
| | Add Dextran RapidSpheres™ to sample and mix. | 60 µL/mL of sample NO INCUBATION, proceed immediately to next step | 60 µL/mL of sample NO INCUBATION, proceed immediately to next step |
| 14 | 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 1.25 mL for samples < 1 mL<br>• Top up to 2.5 mL for samples ≥ 1 mL | • Top up to 2.5 mL for samples < 2 mL<br>• Top up to 5 mL for samples ≥ 2 - 4 mL<br>• Top up to 10 mL for samples > 4 mL |
| | Place the tube (without lid) into the magnet and incubate. | RT for 5 minutes | RT for 5 minutes |
| 15 | 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 |
| 16 | Remove the tube from the magnet and place the new tube (without lid) into the magnet and incubate for a second separation. | RT for 1 minute | RT for 1 minute |
| 17 | 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 | 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.

**Table 2. EasySep™ Human IgG+ Memory B Cell Isolation Kit Protocol**

| | | EASYSEP™ MAGNETS |
|---|---|---|
| STEP | INSTRUCTIONS | Easy 50 (Catalog #18002)  |
| 1 | Prepare sample at the indicated cell concentration within the volume range. | 1 x 10^8 cells/mL<br>10 - 25 mL |
| | Add sample to required tube. | 50 mL (30 x 115 mm) conical tube<br>(e.g. Catalog #38010) |
| 2 | Add CD27 Positive Selection Cocktail to sample. | 50 µL/mL of sample |
| | Mix and incubate. | RT for 3 minutes |
| 3 | Vortex Releasable RapidSpheres™.<br>NOTE: Particles should appear evenly dispersed. | 30 seconds |
| | Add Releasable RapidSpheres™ to sample and mix. | 100 µL/mL of sample |
| 4 | Add Memory B Cell Pre-Enrichment Cocktail to sample. | 100 µL/mL of sample |
| | Mix and incubate. | 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 25 mL for samples ≤ 10 mL<br>• Top up to 50 mL for samples > 10 mL |
| | Place the tube (without lid) into the magnet and incubate. | RT for 10 minutes |
| 6 | Carefully pipette** (do not pour) the supernatant into a new tube. | Use a new 50 mL tube<br>Set aside supernatant for isolating naïve B cells (Table 4) if desired |
| 7 | Remove the tube from the magnet and 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 25 mL for samples ≤ 10 mL<br>• Top up to 50 mL for samples > 10 mL |
| | Place the tube (without lid) into the magnet and incubate. | RT for 10 minutes |
| 8 | Carefully pipette** (do not pour) off the supernatant. | Discard supernatant |
| 9 | Repeat steps as indicated. | Steps 7 and 8, two more times<br>(total of 4 x 10-minute separations) |
| 10 | Remove the tube from the magnet and add recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times. Be sure to collect cells off the sides of the tube. | Same volume as the original starting sample volume (i.e. same volume used in step 1) |
| 11 | Add Release Buffer to sample. | 100 µL/mL of sample |
| | Mix and incubate. | RT for 3 minutes |
| 12 | Add IgM/IgD/IgA Depletion Cocktail to sample. | 100 µL/mL of sample |
| | Mix and incubate. | RT for 5 minutes |
| 13 | Vortex Dextran RapidSpheres™.<br>NOTE: Particles should appear evenly dispersed. | 30 seconds |
| 14 | Add Dextran RapidSpheres™ to sample. | 60 µL/mL of sample |
| | Mix and incubate. | RT for 3 minutes |
| 15 | 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 25 mL for samples ≤ 10 mL<br>• Top up to 50 mL for samples > 10 mL |
| | Place the tube (without lid) into the magnet and incubate. | RT for 10 minutes |
| 16 | Carefully pipette** (do not pour) the enriched cell suspension into a new tube. | Use a new 50 mL tube |
| 17 | Remove the tube from the magnet and place the new tube (without lid) into the magnet and incubate for a second separation. | RT for 5 minutes |
| 18 | Carefully pipette** (do not pour) the enriched cell suspension into a new tube. | 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.

After step 6 of a manual EasySep™ Human IgG+ Memory B Cell Isolation Kit Protocol (see Tables 1 and 2), the supernatant can be further enriched to obtain naïve (CD19+CD27-) B cells. Refer to Tables 3 and 4 for detailed instructions regarding the EasySep™ procedure for each magnet.

**Table 3. Optional: Human Naïve B Cell Enrichment Protocol**

| | | EASYSEP™ MAGNETS | |
|---|---|---|---|
| STEP | INSTRUCTIONS |  **EasySep™ (Catalog #18000)** | **"The Big Easy" (Catalog #18001)**  |
| 1 | **Ensure cells are placed in the required tube.** | Supernatant from Table 1, step 6 must be in a 5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007) | Supernatant from Table 1, step 6 must be in a 14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008) |
| 2 | **Vortex Dextran RapidSpheres™.** **NOTE: Particles should appear evenly dispersed.** | 30 seconds | 30 seconds |
| | **Add Dextran RapidSpheres™ to sample and mix.** | 40 µL/mL of original sample volume (see Table 1, step 1) NO INCUBATION, proceed immediately to next step | 40 µL/mL of original sample volume (see Table 1, step 1) NO INCUBATION, proceed immediately to next step |
| 3 | **Place the tube (without lid) into the magnet and incubate.** | RT for 5 minutes | RT for 5 minutes |
| 4 | **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 | 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.

**Table 4. Optional: Human Naïve B Cell Enrichment Protocol**

| | | EASYSEP™ MAGNETS |
|---|---|---|
| STEP | INSTRUCTIONS | **Easy 50 (Catalog #18002)**  |
| 1 | **Ensure cells are placed in the required tube.** | Supernatant from Table 2, step 6 must be in a 50 mL (30 x 115 mm) conical tube (e.g. Catalog #38010) |
| 2 | **Vortex Dextran RapidSpheres™.** **NOTE: Particles should appear evenly dispersed.** | 30 seconds |
| 3 | **Add Dextran RapidSpheres™ to sample.** | 40 µL/mL of original sample volume (see Table 2, step 1) |
| | **Mix and incubate.** | RT for 3 minutes |
| 4 | **Place the tube (without lid) into the magnet and incubate.** | RT for 10 minutes |
| 5 | **Carefully pipette** (do not pour) the enriched cell suspension into a new tube.** | Isolated cells are ready for use |

RT - room temperature (15 - 25°C)
** Collect the entire supernatant, all at once, into a single pipette.

## Notes and Tips

ASSESSING PURITY

For purity assessment of CD19+CD27+IgG+ B cells by flow cytometry, use the following fluorochrome-conjugated antibody clones:

- Anti-Human CD19 Antibody, Clone HIB19 (Catalog #60005), and
- Anti-human IgG antibody, clone G18-145
- Optional: Anti-Mouse CD27 Antibody, Clone LG.3A10 (Catalog #60160), which cross-reacts with human CD27

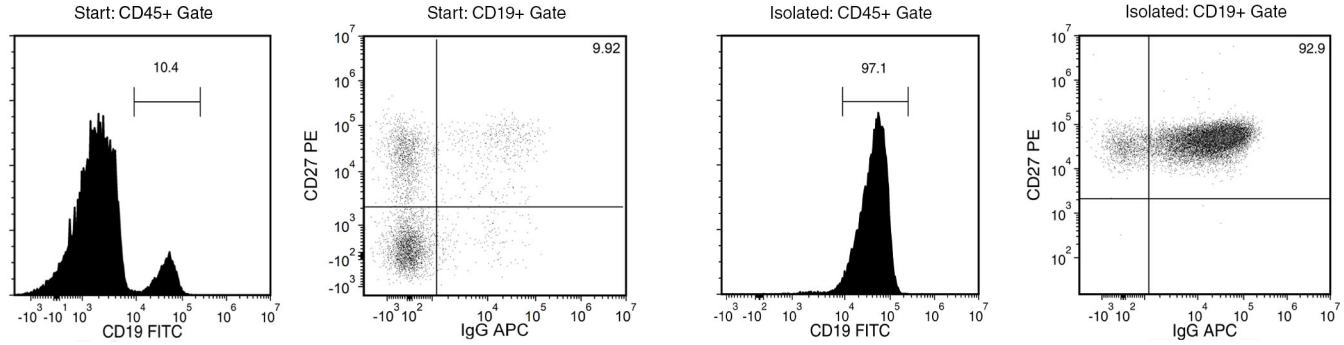
NOTE: Brilliant Violet™ antibody conjugates should be carefully titrated on EasySep™ Release-isolated cells prior to analysis by flow cytometry or fluorescence microscopy. For purity assessment with Brilliant Violet™ antibody conjugates, use of BD Horizon Brilliant™ Stain Buffer is recommended to reduce non-specific interactions. For more information, refer to the manufacturer's instructions or contact us at techsupport@stemcell.com.

ALTERNATIVE PROTOCOLS

For the following isolation protocols, contact us at techsupport@stemcell.com:

- Isolation of total IgG+ B cells without CD27 positive selection
- Isolation of human IgG+ memory B cells using EasyEights™ EasySep™ Magnet (Catalog #18103)

## Data




Starting with PBMCs, the IgG+ memory B cell content (CD19+CD27+IgG+) of the isolated fraction is typically 82.6 ± 7.0% (mean ± SD using “The Big Easy” EasySep™ Magnet). In the above example, the purities of the start and isolated fractions are 1.0% and 90.2% respectively.