# EasySep™ Release Human Biotin Positive Selection Kit or EasySep™ Release Mouse Biotin Positive Selection Kit



Catalog #17653 Catalog #17655 For processing 1 x 10<sup>9</sup> cells For processing 1 x 10<sup>9</sup> cells

Positive Selection

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# Description

Isolate highly purified cells labeled with biotinylated antibodies from fresh or previously frozen human peripheral blood mononuclear cells (PBMCs), washed leukapheresis samples, or mouse splenocytes.

- · Highly purified cells labeled with biotinylated antibodies isolated from human or mouse tissues in less than 40 minutes
- No-wash removal of EasySep™ Releasable RapidSpheres™

These kits target cells labeled with biotinylated antibodies (not provided) for positive selection with antibody complexes recognizing biotin and EasySep™ Releasable RapidSpheres™. Desired cells are labeled with antibodies and magnetic particles, and separated without columns using an EasySep™ magnet. Unwanted cells are simply poured off, while desired cells remain in the tube. Then, bound magnetic particles are removed from the EasySep™-isolated, biotin-antibody labeled cells, which are immediately available for downstream applications such as flow cytometry, culture, or DNA/RNA extraction. Following cell isolation with these EasySep™ Release kits, 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™ Release Biotin<br>Positive Selection Cocktail                                     | 17653C      | 1 x 0.5 mL | Store at 2 - 8°C.<br>Do not freeze. | Stable until expiry date (EXP) on label. | A combination of monoclonal antibodies in PBS and 0.1% BSA.                               |
| EasySep™ Releasable<br>RapidSpheres™ 50201   | 50201       | 1 x 1 mL   | Store at 2 - 8°C.<br>Do not freeze. | Stable until expiry date (EXP) on label. | A suspension of magnetic particles in water.  |
| EasySep™ Release Buffer<br>(Concentrate)   | 20165       | 3 x 1 mL   | Store at 2 - 8°C.<br>Do not freeze. | Stable until expiry date (EXP) on label. | A buffer for release of Releasable RapidSpheres™ from cells following positive selection. |
| EasySep <sup>TM</sup> Anti-Human CD32<br>(Fc gamma RII) Blocker for<br>Positive Selection* | 18520       | 1 x 1 mL   | Store at 2 - 8°C.<br>Do not freeze. | Stable until expiry date (EXP) on label. | A combination of monoclonal antibodies in PBS.  |
| OR<br>Normal Rat Serum**   | 13551       | 1 x 2 mL   | Store at -20°C.                     | Stable until expiry date (EXP) on label. | Mycoplasma-free normal rat serum.   |

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.

# Additional Reagent Stability Information

| REAGENT NAME              | STORAGE           | SHELF LIFE  |
|---------------------------|-------------------|---|
| Normal Rat Serum (in-use) | Store at 2 - 8°C. | Stable for at least 2 months. Do not exceed expiry date (EXP) on label. |

<sup>\*</sup> Supplied only with EasySep™ Release Human Biotin Positive Selection Kit (Catalog #17653)

<sup>\*\*</sup> Supplied only with EasySep™ Release Mouse Biotin Positive Selection Kit (Catalog #17655)

### EasySep™ Release Human Biotin Positive Selection Kit or EasySep™ Release Mouse Biotin Positive Selection Kit



## Sample Preparation

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

#### **HUMAN PERIPHERAL BLOOD**

Prepare a PBMC suspension from whole blood by centrifugation over a density gradient medium (e.g. Lymphoprep<sup>™</sup>, Catalog #07801). For more rapid PBMC preparation, use the SepMate<sup>™</sup> RUO (Catalog #86450/86415) or SepMate<sup>™</sup> 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 (e.g. Catalog #27250) for optimal results.

After preparation, resuspend cells at 1 x 10<sup>8</sup> 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).

#### **HUMAN LEUKAPHERESIS**

Wash the peripheral blood leukapheresis sample by adding an equivalent volume of recommended medium or PBS containing 2% fetal bovine serum (FBS). Centrifuge at 500 x g for 10 minutes at room temperature (15 - 25°C). If red blood cell (RBC) lysis is necessary, lyse with Ammonium Chloride Solution (Catalog #07800). If platelet removal is necessary, centrifuge at 120 x g for 10 minutes with the brake off. Remove the supernatant and resuspend the cells at 1 x 10^8 cells/mL in recommended medium.

#### MOUSE SPLEEN

Disrupt spleen in recommended medium. Remove aggregates and debris by passing cell suspension through a 70 µm mesh nylon strainer (e.g. Catalog #27216). Centrifuge at 300 x g for 10 minutes and resuspend at 1 x 10^8 nucleated cells/mL in recommended medium.

Ammonium chloride treatment is not recommended when preparing the cells for separation.

#### OTHER SAMPLE SOURCES

If using other sample sources or tissues, contact us at techsupport@stemcell.com for more information.

#### Recommended Medium

EasySep™ Buffer (Catalog #20144), 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 Tables 1 and 2 for detailed instructions regarding the EasySep™ procedure for each magnet.

Table 1. EasySep™ Release Human Biotin Positive Selection Kit or EasySep™ Release Mouse Biotin Positive Selection Kit Protocol

|   |   |   | EASYSEP™ MAGNETS  |   |  |  |  |
|---|---|---|---|---|--|--|--|
| Thirts release buffer (XI).  NOTE: Release buffer (IX) must be prepared on the day of use. Refer to step 12 for required volume.  Refer to step 12 for required volume.  1 x 10°8 cells/mL 0.28 - 2 mL 0.25 - 2 m | STEP INSTRUCTIONS   |   |   |   |  |  |  |
| Add sample to required tube.    Solution    | 1   |   | NOTE: Release buffer (1X) must be prepared on the day of use.         | NOTE: Release buffer (1X) must be prepared on the day of use.   |  |  |  |
| If isolating mouse cells (Catalog #17655), add Rat Serum to sample.  If isolating human cells (Catalog #17655), add If isolating human cells (Catalog #17653), add FeR blocker to sample.  Add biolinylated antibody to sample.*  OPTIONAL WASH STEP may improve performance. Add recommended medium to top up the sample in original volume.  Add Selection Cocktail to sample.*  OVOTES: Do not vortex cocktail.  Mix and incubate.  Add RapidSpheres™.  NOTE: Particles should appear evenly dispersed.  Add recommended medium to top up the sample.  Add RapidSpheres™ to sample.  Add recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2-3 times.  Place the tube (without lid) into the magnet and incubate.  RT for 5 minutes  RT for 5 minutes  RT for 5 minutes   | 2   | Prepare sample at the indicated cell concentration within the volume range. |   |   |  |  |  |
| Add second commended medium to top up the sample in original volume.  Add Selection Cocktail to sample.  Add Selection Cocktail to sample.  Add Selection Cocktail to sample.  Add Repolichere.  Add Repolichere.  Add Repolichere.  Add Repolichere.  Add recommended medium and centrifuge at oliceard supernatant. Resuspend in the same volume as step 2.  Add Selection Cocktail to sample.  Add Selection Cocktail to sample.  Add Repolichere.  Add Repolichere.  Add Repolichere.  Add Repolichere.  Add Repolichere.  Add Selection Cocktail to sample.  Add Selection Cocktail to sample.  Add Repolichere.  Add recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2-3 times.  Add recommended medium to top up the sample.  Add recommended medium to top up the sample.  Add recommended medium and centrifuge at 700 pt p | 3   | Add 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<br>(e.g. Catalog #38008)   |  |  |  |
| Mix and incubate.  OPTIONAL WASH STEP may improve performance. Add recommended medium to top up the sample to the indicated volume and centrifuge. Resuspend sample in original volume.  Add Selection Cocktail to sample.**  NOTE: Do not vortex cocktail.  Mix and incubate.  7 Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.  8 Add RapidSpheres™ to sample.  Mix and incubate.  Add recommended medium to top up the sample in original volume.  8 Add RapidSpheres™ to sample.  Add Selection Cocktail to sample.**  100 µL/mL of sample  25 - 100 µL/mL of sample  RT for 3 minutes  RT for 3 minutes  RT for 3 minutes  100 µL/mL of sample  | 4   | Rat Serum to sample.  OR  If isolating human cells (Catalog #17653), add    | OR  | OR  |  |  |  |
| Mix and incubate.  OPTIONAL WASH STEP may improve performance. Add recommended medium to top up the sample to the indicated volume and centrifuge. Resuspend sample in original volume.  Top up with 2-fold excess recommended medium and centrifuge at 300 x g for 10 minutes at RT with low brake. Carefully aspirate and discard supernatant. Resuspend in the same volume as step 2.  Add Selection Cocktail to sample.**  NOTE: Do not vortex cocktail.  Mix and incubate.  Top up with 2-fold excess recommended medium and centrifuge at 300 x g for 10 minutes at RT with low brake. Carefully aspirate and discard supernatant. Resuspend in the same volume as step 2.  25 - 100 μL/mL of sample  25 - 100 μL/mL of sample  RT for 3 minutes  RT for 3 minutes  RT for 3 minutes  RT for 3 minutes  30 seconds  30 seconds  30 seconds  RT for 3 minutes  RT for 5 minutes  RT for 5 minutes  RT for 5 minutes  |   | Add biotinylated antibody to sample.*                                       | 0.25 - 2 μg/mL of sample  | 0.25 - 2 μg/mL of sample  |  |  |  |
| Add recommended medium to top up the sample to the indicated volume and centrifuge. Resuspend sample in original volume.  Add Selection Cocktail to sample.**  NOTE: Do not vortex cocktail.  Mix and incubate.  7 Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.  Add RapidSpheres™ to sample.  Add recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.  Place the tube (without lid) into the magnet and incubate.  RT for 5 minutes   | 5   | Mix and incubate.   | RT for 5 minutes  | RT for 5 minutes  |  |  |  |
| NOTE: Do not vortex cocktail.  Mix and incubate.  RT for 3 minutes  30 seconds  30 seconds  Add RapidSpheres™ to sample.  Mix and incubate.  RT for 3 minutes  RT for 5 minutes  RT for 5 minutes  RT for 5 minutes  RT for 5 minutes   | Add recommended medium to top up the sample to the indicated volume and centrifuge. Resuspend sample in |   | 300 x g for 10 minutes at RT with low brake. Carefully aspirate and   | Top up with 2-fold excess recommended medium and centrifuge at 300 x g for 10 minutes at RT with low brake. Carefully aspirate and discard supernatant. Resuspend in the same volume as step 2. |  |  |  |
| Mix and incubate.       RT for 3 minutes         7       Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.       30 seconds         8       Add RapidSpheres™ to sample.       100 μL/mL of sample       100 μL/mL of sample         Mix and incubate.       RT for 3 minutes       RT for 3 minutes         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 < 4 mL         Place the tube (without lid) into the magnet and incubate.       RT for 5 minutes       RT for 5 minutes  | 6   | •   | 25 - 100 μL/mL of sample  | 25 - 100 μL/mL of sample  |  |  |  |
| NOTE: Particles should appear evenly dispersed.  8 Add RapidSpheres™ to sample.  Mix and incubate.  RT for 3 minutes  RT for 3 minutes  Add recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.  Place the tube (without lid) into the magnet and incubate.  RT for 5 minutes   |   | Mix and incubate.   | RT for 3 minutes  | RT for 3 minutes  |  |  |  |
| Mix and incubate.  RT for 3 minutes  RT for 3 minutes  RT for 3 minutes  RT for 3 minutes  Place the tube (without lid) into the magnet and incubate.  RT for 3 minutes  RT for 3 minutes  Top up to 2.5 mL  Top up to 2.5 mL  Place the tube (without lid) into the magnet and incubate.  RT for 5 minutes  RT for 3 minutes  **RT for 3 minutes  **RT for 3 minutes  **RT for 5 minutes  RT for 5 minutes   | 7   |   | 30 seconds  | 30 seconds  |  |  |  |
| Mix and incubate.  Add recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.  Place the tube (without lid) into the magnet and incubate.  RT for 3 minutes  • Top up to 5 mL for samples < 4 mL  • Top up to 10 mL for samples ≥ 4 mL  RT for 5 minutes  RT for 5 minutes   | ,   | Add RapidSpheres™ to sample.  | 100 μL/mL of sample   | 100 μL/mL of sample   |  |  |  |
| to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.  Top up to 2.5 mL  Top up to 2.5 mL  Top up to 10 mL for samples ≥ 4 mL  Place the tube (without lid) into the magnet and incubate.  RT for 5 minutes   |   | Mix and incubate.   | RT for 3 minutes  | RT for 3 minutes  |  |  |  |
| Place the tube (without lid) into the magnet and incubate.  RT for 5 minutes  RT for 5 minutes  | 9   | to the indicated volume. Mix by gently pipetting                            | Top up to 2.5 mL  |   |  |  |  |
| Continue to step 10, next page Continue to step 10, next page Continue to step 10, next page  |   |   | RT for 5 minutes  | RT for 5 minutes  |  |  |  |
|   |   | Continue to step 10, next page  | Continue to step 10, next page  | Continue to step 10, next page  |  |  |  |



|      |  | EASYSEP™ MAGNETS   |  |  |  |  |
|------|--|--|--|--|--|--|
| STEP | INSTRUCTIONS (CONTINUED)   | EasySep™<br>(Catalog #18000)                                       | "The Big Easy"<br>(Catalog #18001)   |  |  |  |
| 10   | Pick up the magnet, and in one continuous motion invert the magnet and tube,‡ pouring off the supernatant. Remove the tube from the magnet; this tube contains the isolated cells. | Discard supernatant  | Discard supernatant  |  |  |  |
| 11   | Repeat steps as indicated.   | Steps 9 and 10, two more times (total of 3 x 5-minute separations) | Steps 9 and 10, two more times<br>(total of 3 x 5-minute separations)  |  |  |  |
| 12   | Add release buffer (1X) 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   | <ul> <li>Top up to 5 mL for start sample &lt; 4 mL</li> <li>Top up to 10 mL for start sample ≥ 4 mL</li> </ul> |  |  |  |
|      | Incubate.  | RT for 3 minutes   | RT for 3 minutes   |  |  |  |
| 13   | Place the tube (without lid) into the magnet and incubate.   | RT for 5 minutes   | RT for 5 minutes   |  |  |  |
| 14   | Pick up the magnet, and in one continuous motion invert the magnet and tube, <sup>‡</sup> pouring the enriched cell suspension into a new tube.                                    | Isolated cells (in the new tube) are ready for use                 | Isolated cells (in the new tube) are ready for use   |  |  |  |

RT - room temperature (15 - 25°C)

\* Titrate biotinylated antibody for optimal purity and recovery. Contact us at techsupport@stemcell.com for more information.

\*\*\* Titrate EasySep™ Release Biotin Positive Selection Cocktail for optimal purity and recovery. Contact us at techsupport@stemcell.com for more information.

‡ 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™ Release Human Biotin Positive Selection Kit or EasySep™ Release Mouse Biotin Positive Selection Kit Protocol

|  |  | EASYSEP™ MAGNETS   |   |   |  |   |
|--|--|--|---|---|--|---|
|  |  | EasyPlate <sup>™</sup>   |   | EasyEights™ (C  | atalog #18103)   |   |
| STEP   | INSTRUCTIONS   | (Catalog #18102)   |   | 5 mL tube   | 14 mL tube   | 111111111111111111111111111111111111111 |
| 1  | Dilute Release Buffer (Concentrate) to prepare release buffer (1X).  | Dilute 1 in 40 with recommended medium.<br>NOTE: Release buffer (1X) must be<br>prepared on the day of use.<br>Refer to step 12 for required volume.   | NOTE: I   | 40 with recommended medium.<br>Release buffer (1X) must be<br>pared on the day of use.<br>step 12 for required volume.  | Dilute 1 in 40 with recommended medium.<br>NOTE: Release buffer (1X) must be<br>prepared on the day of use.<br>Refer to step 12 for required volume.                                 |   |
| 2  | Prepare sample at the indicated cell concentration within the volume range.  | 1 x 10^8 cells/mL<br>0.05 - 0.2 mL   | 1 x 10^8 cells/mL<br>0.25 - 2 mL<br>1 x 10^8 cells/mL<br>0.5 - 8 mL |   |  |   |
| 3  | Add sample to required tube (or plate when using the EasyPlate™ EasySep™ Magnet).  | Round-bottom, non-tissue culture-treated<br>96-well plate<br>(e.g. Catalog #38018)   | polystyrene round-bottom tube polystyrene round-bottom              |   | 14 mL (17 x 95 mm)<br>polystyrene round-bottom tube<br>(e.g. Catalog #38008)   | 9                                       |
| 4  | If isolating mouse cells (Catalog #17655), add<br>Rat Serum to sample.<br>OR<br>If isolating human cells (Catalog #17653), add<br>FcR blocker to sample. | 50 μL/mL of sample<br>OR<br>100 μL/mL of sample  |   | 50 μL/mL of sample<br>OR<br>100 μL/mL of sample   | 50 μL/mL of sample<br>OR<br>100 μL/mL of sample  |   |
|  | Add biotinylated antibody to sample.*  | 0.25 - 2 μg/mL of sample   | 0.2   | 25 - 2 μg/mL of sample  | 0.25 - 2 μg/mL of sample   |   |
| 5  | Mix and incubate.  | RT for 5 minutes   |   | RT for 5 minutes  | RT for 5 minutes   |   |
| OPTIONAL WASH STEP may improve performance. Add recommended medium to top up the sample to the indicated volume and centrifuge. Resuspend sample in original volume. |  | Top up with 2-fold excess recommended medium and centrifuge at 300 x g for 10 minutes at RT with low brake. Carefully aspirate and discard supernatant.  Resuspend in the same volume as step 2. | medium<br>10 minutes<br>aspirat                                     | th 2-fold excess recommended<br>and centrifuge at 300 x g for<br>at RT with low brake. Carefully<br>te and discard supernatant.<br>If in the same volume as step 2. | Top up with 2-fold excess recomme<br>medium and centrifuge at 300 x g<br>10 minutes at RT with low brake. Ca<br>aspirate and discard supernatar<br>Resuspend in the same volume as s | g for<br>arefully<br>nt.                |
|  | Add Selection Cocktail to sample.**  NOTE: Do not vortex cocktail.   | 25 - 100 μL/mL of sample   | 25 - 100 μL/mL of sample  |   | 25 - 100 μL/mL of sample   |   |
| 6  | Mix and incubate.  | RT for 3 minutes   | RT for 3 minutes  |   | RT for 3 minutes   |   |
| 7  | Vortex Releasable RapidSpheres™.<br>NOTE: Particles should appear evenly dispersed.  | 30 seconds   | 30 seconds 30 seconds   |   | 30 seconds   |   |
|  | Add Releasable RapidSpheres™ to sample.  | 100 μL/mL of sample  | -   | 100 μL/mL of sample   | 100 μL/mL of sample  |   |
| 8  | Mix and incubate.  | RT for 3 minutes   |   | RT for 3 minutes  | RT for 3 minutes   |   |
| 9  | Add recommended medium to top up sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.  | Top up to 0.25 mL  | I ON UN TO 25 MI  |   | <ul> <li>Top up to 5 mL for samples &lt; 4 mL</li> <li>Top up to 10 mL for samples ≥ 4 mL</li> </ul>   |   |
|  | Place the tube or plate (without lid) into the magnet and incubate.  | RT for 5 minutes   | RT for 10 minutes <sup>‡</sup> RT for 10 minute                     |   | RT for 10 minutes <sup>‡</sup>   |   |
|  | Continue to step 10, next page   | Continue to step 10, next page   | Conti   | nue to step 10, next page   | Continue to step 10, next page   | Э                                       |



|      |   | EASYSEP™ MAGNETS   |   |   |  |   |  |
|------|---|--|---|---|--|---|--|
|      | INSTRUCTIONS (CONTINUED)  | EasyPlate™<br>(Catalog #18102)                                     |   | EasyEights™ (Catalog #18103)                    |  |   |  |
| STEP |   |  |   | 5 mL tube                                       | 14 mL tube   |   |  |
| 10   | Carefully pipette*** (do not pour) off the supernatant. Remove the tube or plate, containing the isolated cells, from the magnet. | Discard supernatant  | Discard supernatant   |   | Discard supernatant  |   |  |
| 11   | Repeat steps as indicated.  | Steps 9 and 10, two more times (total of 3 x 5-minute separations) | Steps 9 and 10, two more times (total of 3 x 10-minute separations) |   | Steps 9 and 10, two more times (total of 3 x 10-minute separations)      |   |  |
| 12   | Add release buffer (1X) to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.            | Top up to 0.25 mL  | Top up to 2.5 mL  |   | <ul><li>Top up to 5 mL for sam</li><li>Top up to 10 mL for san</li></ul> | • |  |
|      | Mix and incubate.   | RT for 3 minutes   | RT for 3 minutes  |   | RT for 3 minutes   |   |  |
| 13   | Place the tube or plate (without lid) into the magnet and incubate.   | RT for 5 minutes   |   | RT for 10 minutes <sup>‡</sup>                  | RT for 10 minutes <sup>‡</sup>   |   |  |
| 14   | Carefully pipette*** (do not pour) the enriched cell suspension into a new tube.  | Isolated cells (in the new tube)<br>are ready for use              | Isolate   | ed cells in (the new tube)<br>are ready for use | Isolated cells (in the new tube) are ready for use                       |   |  |

RT - room temperature (15 - 25°C)

\* Titrate biotinylated antibody for optimal purity and recovery. Contact us at techsupport@stemcell.com for more information.

\*\* Titrate EasySep™ Release Biotin Positive Selection Cocktail for optimal purity and recovery. Contact us at techsupport@stemcell.com for more information.

‡ Incubation time may be reduced to 5 minutes for some samples.

\*\*\* Collect the entire supernatant, 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]).

### EasySep™ Release Human Biotin Positive Selection Kit or EasySep™ Release Mouse Biotin Positive Selection Kit



### Notes and Tips

#### EASYSEP™ RELEASE BUFFER

EasySep™ Release Buffer (Concentrate) is supplied as a 40X concentrate; release buffer (1X) must be prepared on the day of use. To prepare release buffer (1X), dilute an appropriate volume 1 in 40 with recommended medium. Refer to step 12 of Table 1 or Table 2 for required volume.

#### OPTIMIZING PURITY AND RECOVERY

In some cases, titration of the biotinylated antibody (not provided) and EasySep<sup>TM</sup> Release Biotin Positive Selection Cocktail may be required to achieve optimal purity and recovery. Contact us at techsupport@stemcell.com for more information.

Recovery of positively selected cells is also dependent on the quality of biotinylated antibody (not provided) used for positive selection. Antibodies that have expired or that have been stored improperly may show lower affinity for the surface marker on the target cell, resulting in lower recovery.

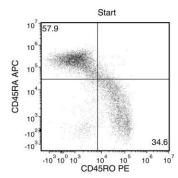
#### ASSESSING PURITY

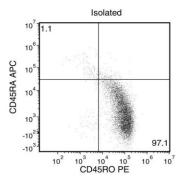
For purity assessment of biotinylated cells by flow cytometry, use one of the following methods:

- Add fluorochrome-conjugated antibody to label the selected cells.
- NOTE: The biotinylated antibody may block the labeling antibody.
- Use fluorochrome-conjugated antibodies to alternative cell surface markers.
- · Use a fluorochrome-conjugated secondary antibody, such as Goat Anti-Mouse IgG (H+L) Antibody, Polyclonal (Catalog #60138).

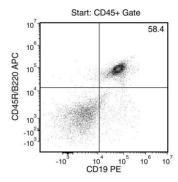
NOTE: Brilliant Violet™ antibody conjugates should be carefully titrated on EasySep™ Release-isolated cells prior to analysis by flow cytometry or fluorescence microscopy. For purity assessment with Brilliant Violet™ antibody conjugates, use of BD Horizon Brilliant™ Stain Buffer is recommended to reduce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

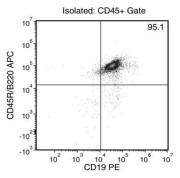
### Data





Starting with fresh PBMCs, the purities of the start and final isolated fractions are 34.6% and 97.1%, respectively, using a biotinylated anti-human CD45RO antibody and EasySep™ Release Human Biotin Positive Selection Kit (as assessed by labeling with CD45RO and CD45RA).





Starting with mouse splenocytes, the purities of the start and final isolated fractions are 58.4% and 95.1%, respectively, using a biotinylated anti-mouse CD19 antibody and EasySep™ Release Mouse Biotin Positive Selection Kit (as assessed by labeling with CD19 and CD45R/B220).

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