EasySep™ Release Human PE Positive Selection Kit or EasySep™ Release Mouse PE Positive Selection Kit

Catalog #17654 For processing 1 x 10^9 cells
Catalog #17656 For processing 1 x 10^9 cells

Negative Selection

Document #10000005223 | Version 02

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Description

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

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

This kit targets cells labeled with PE-conjugated antibodies (not provided) for positive selection with antibody complexes recognizing PE 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, PE antibody-labeled cells, which are immediately available for downstream applications such as flow cytometry, culture, or DNA/RNA extraction. 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™ Release PE Positive Selection Cocktail	17654C	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	1 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 (Concentrate)	20165	3 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.
EasySep™ Anti-Human CD32 (Fc gamma RII) Blocker for Positive Selection*	18520	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.
OR 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

* Supplied only with EasySep™ Release Human PE Positive Selection Kit (Catalog #17654)
** Supplied only with EasySep™ Release Mouse PE Positive Selection Kit (Catalog #17656)

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.

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™, 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 (e.g 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).

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 pages 1 and 2 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 PE Positive Selection Kit or EasySep™ Release Mouse PE Positive Selection Kit Protocol

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	 **EasySep™ (Catalog #18000)**	**"The Big Easy" (Catalog #18001)** 
1	Dilute Release Buffer (Concentrate) to prepare release buffer (1X).	Dilute 1 in 40 with recommended medium. NOTE: Release buffer (1X) must be prepared on the day of use. Refer to step 12 for required volume.	Dilute 1 in 40 with recommended medium. NOTE: Release buffer (1X) must be prepared on the day of use. 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 0.25 - 2 mL	1 x 10^8 cells/mL 0.5 - 8 mL
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 (e.g. Catalog #38008)
4	If isolating mouse cells (Catalog #17656), add Rat Serum to sample. OR If isolating human cells (Catalog #17654), add FcR blocker to sample.	50 µL/mL of sample OR 100 µL/mL of sample	50 µL/mL of sample OR 100 µL/mL of sample
5	Add PE-conjugated antibody to sample.*	0.5 - 2 µg/mL of sample	0.5 - 2 µg/mL of sample
	Mix and incubate.	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.	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.
6	Add Selection Cocktail to sample.** NOTE: Do not vortex cocktail.	25 - 100 µL/mL of sample	25 - 100 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 minutes
7	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	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
9	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 • 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
Continue to step 10, next page		Continue to step 10, next page	Continue to step 10, next page

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		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS (CONTINUED)	 **EasySep™ (Catalog #18000)**	**"The Big Easy" (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 (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	• Top up to 5 mL for start sample ≤ 4 mL • Top up to 10 mL for start sample > 4 mL
	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,‡ 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 PE-conjugated antibody for optimal purity and recovery. Contact us at techsupport@stemcell.com for more information.
** Titrate EasySep™ Release PE 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 PE Positive Selection Kit or EasySep™ Release Mouse PE Positive Selection Kit Protocol

		EASYSEP™ MAGNETS		
STEP	INSTRUCTIONS	EasyPlate™ (Catalog #18102)	EasyEights™ (Catalog #18103) 5 mL tube	EasyEights™ (Catalog #18103) 14 mL tube
1	Dilute Release Buffer (Concentrate) to prepare release buffer (1X).	Dilute 1 in 40 with recommended medium. NOTE: Release buffer (1X) must be prepared on the day of use. Refer to step 12 for required volume.	Dilute 1 in 40 with recommended medium. NOTE: Release buffer (1X) must be prepared on the day of use. Refer to step 12 for required volume.	Dilute 1 in 40 with recommended medium. NOTE: Release buffer (1X) must be prepared on the day of use. 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 0.05 - 0.2 mL	1 x 10^8 cells/mL 0.25 - 2 mL	1 x 10^8 cells/mL 0.5 - 8 mL
3	Add sample to required tube (or plate when using the EasyPlate™ EasySep™ Magnet).	Round-bottom, non-tissue culture-treated 96-well plate (e.g. Catalog #38018)	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)
4	If isolating mouse cells (Catalog #17656), add Rat Serum to sample. OR If isolating human cells (Catalog #17654), add FcR blocker to sample.	50 µL/mL of sample OR 100 µL/mL of sample	50 µL/mL of sample OR 100 µL/mL of sample	50 µL/mL of sample OR 100 µL/mL of sample
5	Add PE-conjugated antibody to sample.*	0.5 - 2 µg/mL of sample	0.5 - 2 µg/mL of sample	0.5 - 2 µg/mL of sample
	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.	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.	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.
6	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
	Mix and incubate.	RT for 3 minutes	RT for 3 minutes	RT for 3 minutes
7	Vortex Releasable RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds	30 seconds
8	Add Releasable RapidSpheres™ to sample.	100 µL/mL of sample	100 µL/mL of sample	100 µL/mL of sample
	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	Top up to 2.5 mL	• Top up to 5 mL for samples ≤ 4 mL • Top up to 10 mL for samples > 4 mL
	Place the tube or plate (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 10 minutes‡	RT for 10 minutes‡
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		EASYSEP™ MAGNETS		
STEP	INSTRUCTIONS (CONTINUED)	 **EasyPlate™ (Catalog #18102)**	 **EasyEights™ (Catalog #18103)** **5 mL tube**	**EasyEights™ (Catalog #18103)** **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	• Top up to 5 mL for samples ≤ 4 mL • Top up to 10 mL for samples > 4 mL
	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‡	RT for 10 minutes‡
14	**Carefully pipette*** (do not pour) 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	Isolated cells (in the new tube) are ready for use

RT - room temperature (15 - 25°C)
* Titrate PE-conjugated antibody for optimal purity and recovery. Contact us at techsupport@stemcell.com for more information.
** Titrate EasySep™ Release PE 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]).

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 PE-conjugated antibody (not provided) and EasySep™ Release PE Positive Selection Cocktail may be required to achieve optimal purity and recovery. Contact us at techsupport@stemcell.com for more information.

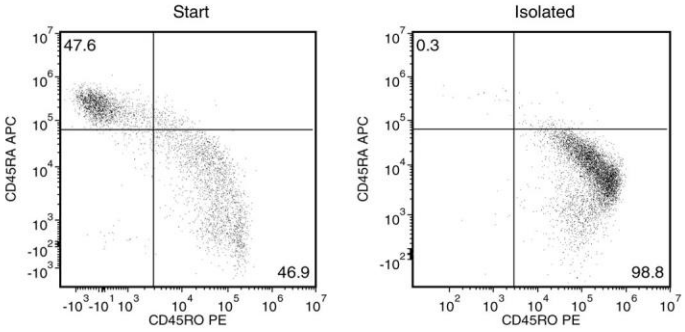
Recovery of positively selected cells is also dependent on the quality of PE-conjugated antibody (not provided) used for positive selection. Antibodies that have expired or that have been stored improperly may show lower affinity for the surface marker on the target cell, resulting in lower recovery.

ASSESSING PURITY

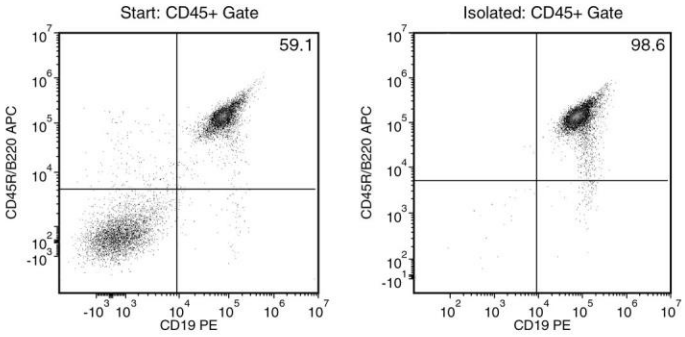
The positively selected cells have already been PE-labeled, so the purity can be assessed directly by flow cytometry.

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 46.9% and 98.8%, respectively, using a PE-conjugated anti-human CD45RO antibody and EasySep™ Release Human PE Positive Selection Kit.



Starting with mouse splenocytes, the purities of the start and final isolated fractions are 59.1% and 98.6%, respectively, using a PE-conjugated anti-mouse CD19 antibody and EasySep™ Release Mouse PE Positive Selection Kit.