

EasySep™ Release Human CD4 Positive Selection Kit

For processing 1 x 10⁹ cells

Catalog #17752

#17752RF RoboSep™

Positive Selection

Document #1000005225 | Version 02



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Description

Isolate highly purified CD4+ cells from fresh or previously frozen peripheral blood mononuclear cells (PBMCs) or washed leukapheresis samples.

- Highly purified human CD4+ cells isolated in less than 30 minutes
- No-wash removal of EasySep™ Releasable RapidSpheres™

This kit targets CD4+ cells for positive selection with antibody complexes recognizing the CD4 surface marker 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 CD4+ 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 Human CD4 Positive Selection Cocktail	17752C	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™ 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.

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.

RoboSep™ Release Human CD4 Positive Selection Kit (17752RF) is supplied with EasySep™ EasyTube™-14 (Catalog #20128) for optimal performance. The use of EasySep™ EasyTube™-14 is not required when performing a manual separation.

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 clumpy suspensions through a 37 µm cell strainer (e.g. Catalog #27250) for optimal results.

After preparation, resuspend cells at 1 x 10⁸ 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

Wash the peripheral blood leukapheresis sample by adding an equivalent volume of recommended medium or PBS containing 2% fetal bovine serum (FBS).

Centrifuge at 300 x g for 10 minutes at room temperature (15 - 25°C). If red blood cell 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 10⁸ cells/mL in recommended medium.



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 CD4 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 9 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 9 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
	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)
3	Add Selection Cocktail to sample. NOTE: Do not vortex cocktail.	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 minutes
4	Vortex Releasable RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
5	Add Releasable RapidSpheres™ to sample.	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 minutes
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	<ul style="list-style-type: none"> • Top up to 5 mL for samples ≤ 3 mL • Top up to 10 mL for samples > 3 mL
	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 off the supernatant. Remove the tube from the magnet; this tube contains the isolated cells.	Discard supernatant	Discard supernatant
8	Repeat steps as indicated.	Steps 6 and 7, two more times (total of 3 x 5-minute separations)	Steps 6 and 7, two more times (total of 3 x 5-minute separations)
9	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 style="list-style-type: none"> • Top up to 5 mL for start sample ≤ 3 mL • Top up to 10 mL for start sample > 3 mL
	Incubate.	RT for 3 minutes	RT for 3 minutes
10	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes
11	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)

* 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 CD4 Positive Selection Kit Protocol

STEP	INSTRUCTIONS	EASYSEP™ MAGNETS		
		 EasyPlate™ (Catalog #18102)	 EasyEights™ (Catalog #18103) 5 mL tube	 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 9 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 9 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 9 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
	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)
3	Add Selection Cocktail to sample. NOTE: Do not vortex cocktail.	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
4	Vortex Releasable RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds	30 seconds
5	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
6	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	<ul style="list-style-type: none"> • Top up to 5 mL for samples ≤ 3 mL • Top up to 10 mL for samples > 3 mL
	Place the tube or plate (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
7	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
8	Repeat steps as indicated.	Steps 6 and 7, two more times (total of 3 x 5-minute separations)	Steps 6 and 7, two more times (total of 3 x 5-minute separations)	Steps 6 and 7, two more times (total of 3 x 5-minute separations)
9	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 style="list-style-type: none"> • Top up to 5 mL for samples ≤ 3 mL • Top up to 10 mL for samples > 3 mL
	Incubate.	RT for 3 minutes	RT for 3 minutes	RT for 3 minutes
10	Place the tube or plate (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
11	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)

** 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]).

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.

Table 3. RoboSep™ Release Human CD4 Positive Selection Kit Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #21000)	
1	Prepare sample at the indicated cell concentration within the volume range.	1 x 10 ⁸ cells/mL 0.5 - 8 mL	
	Add sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)	
2	Select protocol.	<ul style="list-style-type: none"> For samples ≤ 3 mL: Human CD4 Release Positive Selection 17752 - small volume For samples > 3 mL: Human CD4 Release Positive Selection 17752 - large volume 	
3	Vortex Releasable RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	
4	Load the carousel.	Follow on-screen prompts NOTE: When prompted to load a separation tube, place EasySep™ EasyTube™-14 into the magnet.	
	Start the protocol.	Press the green “Run” button	
5	Unload the carousel when the run is complete. Remove the tube containing the isolated cells.	Isolated cells are ready for use	

Notes and Tips

EASYSEPTM 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 9 of Table 1 or Table 2 for required volume.

ASSESSING PURITY

The EasySep™ Release Human CD4 Positive Selection Cocktail uses an anti-CD4 antibody clone that to our knowledge fully or partially blocks all anti-CD4 antibody clones used to assess purity by flow cytometry. For purity assessment of CD4+ cells by flow cytometry, use the following fluorochrome-conjugated antibody clone:

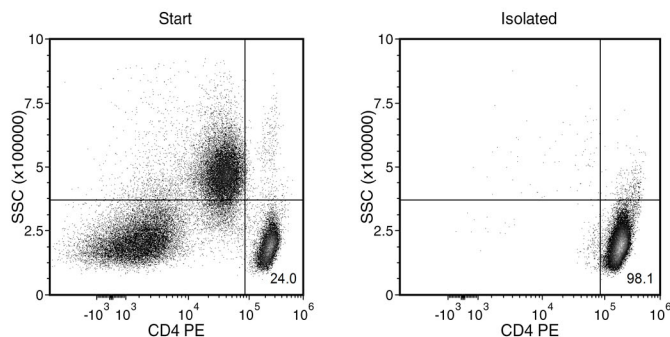
- Anti-Human CD4 Antibody, Clone OKT4 (Catalog #60016; partially blocked)

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.

One of the following methods can also be used:

- Use alternative markers such as fluorochrome-conjugated Anti-Human CD3 Antibody, Clone UCHT1 (Catalog #60011) and Anti-Human CD8a Antibody, Clone RPA-T8 (Catalog #60022) to detect CD3+CD8- cells.
- Use a fluorochrome-conjugated secondary antibody, such as Goat Anti-Mouse IgG (H+L) Antibody, Polyclonal (Catalog #60138).

Data



Starting with a single-cell suspension of human PBMCs, the CD4⁺ T cell content of the isolated fraction is typically $96.1 \pm 4.1\%$ (mean \pm SD using the purple EasySep™ Magnet). In the above example, the purities of the start and final isolated fractions are 24.0% and 98.1%, respectively.

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