



**EasySep™ HLA CD3
Positive Selection Kit**

Positive Selection

Catalog #18051HLA

For processing 1 x 10⁹ cells



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Description

Isolate highly purified CD3+ cells from fresh or previously frozen human peripheral blood mononuclear cells (PBMCs) by immunomagnetic positive selection.

- Fast and easy-to-use
- Up to 99.6% purity
- No columns required

This kit targets CD3+ cells for positive selection with an antibody recognizing the CD3 surface marker. 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. 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™ HLA CD3 Positive Selection Cocktail	18051HC.1	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. Includes an Fc receptor blocking antibody.
EasySep™ Magnetic Nanoparticles Positive Selection	18150H	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™ HLA FCXM Blocking Solution	18210HC	5 x 2 mL	Store at 15 - 25°C.	Stable until expiry date (EXP) on label.*	A solution required for flow cytometric crossmatch analysis following cell isolation.

PBS - phosphate-buffered saline

* Repeated exposure to air may cause some crystallization to occur around the edge of the tube. This crystallization does not affect the performance of the blocking solution in flow cytometric crossmatch analysis.

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 peripheral 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 40 µm Cell Strainer (Catalog #27305) 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).

Recommended Medium

EasySep™ Buffer (Catalog #20144), RoboSep™ Buffer (Catalog #20104), 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 Table 1 for detailed instructions regarding the EasySep™ procedure for each magnet.

Table 1. EasySep™ HLA CD3 Positive Selection 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 0.1 - 2.5 mL NOTE: If starting with fewer than 1 x 10 ⁷ cells, resuspend cells in 0.1 mL.	1 x 10 ⁸ cells/mL 0.25 - 8 mL NOTE: If starting with fewer than 2.5 x 10 ⁷ cells, resuspend cells in 0.25 mL.
	Add sample to required tube.	5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352058)	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)
2	Add Selection Cocktail to sample.	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	RT for 15 minutes	RT for 15 minutes
3	Mix Magnetic Particles. NOTE: Particles should appear evenly dispersed.	Pipette up and down more than 5 times	Pipette up and down more than 5 times
4	Add Magnetic Particles to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 10 minutes	RT for 10 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 2.5 mL	<ul style="list-style-type: none"> Top up to 5 mL for samples < 1 mL Top up to 10 mL for samples ≥ 1 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 off the supernatant. Remove the tube from the magnet; this tube contains the isolated cells.	Discard supernatant	Discard supernatant
7	Repeat steps as indicated.	Steps 5 and 6, two more times (total of 3 x 5-minute separations)	Steps 5 and 6, two more times (total of 3 x 5-minute separations)
8	Resuspend cells in desired medium. Be sure to collect cells from the sides of the tube.	Isolated cells are ready for use	Isolated cells are ready for use
9	OPTIONAL: If proceeding to flow cytometric crossmatch analysis add Blocking Solution.	200 µL	200 µL

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.

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™ HLA CD3 Positive Selection Kit Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #20000 and #21000)
1	Prepare sample at the indicated cell concentration within the volume range.	1 x 10 ⁸ cells/mL 0.25 - 8.5 mL NOTE: If starting with fewer than 2.5 x 10 ⁷ cells, resuspend cells in 0.25 mL.
	Add sample to required tube.	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)
2	Select protocol.	Human CD3 Positive Selection 18051HLA
3	Mix Magnetic Particles. NOTE: Particles should appear evenly dispersed.	Pipette up and down more than 5 times
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. Remove the tube containing the isolated cells and resuspend in desired medium. Be sure to collect cells from the sides of the tube.	Isolated cells are ready for use
6	OPTIONAL: If proceeding to flow cytometric crossmatch analysis add Blocking Solution.	200 µL

Notes and Tips

ASSESSING PURITY

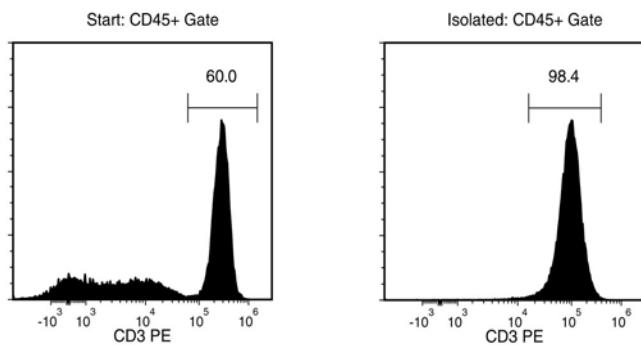
The EasySep™ Human CD3 Positive Selection Cocktail uses an anti-CD3 antibody clone that to our knowledge may block other anti-CD3 antibody clones used to assess purity by flow cytometry. One of the following methods can be used to assess purity:

- Use an alternative marker such as fluorochrome-conjugated Anti-Human CD2 Antibody, Clone RPA-2.10 (Catalog #60007) to detect CD2+ cells.
- Use alternative markers such as fluorochrome-conjugated Anti-Human CD5 Antibody, Clone UCHT2 (Catalog #60082) and Anti-Human CD20 Antibody, Clone 2H7 (Catalog #60008) to detect CD5+CD20- cells.
- Use a fluorochrome-conjugated secondary antibody, such as Goat Anti-Mouse IgG (H+L) Antibody, Polyclonal (Catalog #60138).

CD3+ CELL DEPLETION

The EasySep™ HLA CD3 Positive Selection Kit can also be used to deplete CD3+ cells. Contact us at techsupport@stemcell.com to receive a copy of the protocol.

Data



Starting with PBMCs, the CD3+ cell content of the isolated fraction typically ranges from 94.6 - 99.6% (gated on CD45+ cells). In the above example, the purities of the start and final isolated fractions are 60% and 98.4%, respectively.

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