



## EasySep™ HLA CD2 Positive Selection Kit

Positive Selection

Catalog #18657HLA

For processing  $1 \times 10^9$  cells



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## Description

Isolate highly purified CD2+ cells from fresh or previously frozen human peripheral blood mononuclear cells (PBMCs) by immunomagnetic positive selection.

- Fast and easy-to-use
- Up to 98% purity
- No columns required

This kit targets CD2+ cells for positive selection with an antibody recognizing the CD2 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 CD2 Positive Selection Cocktail	18657HC	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

\* NOTE: 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](http://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 40 µm Cell Strainer (Catalog #27305) for optimal results.

After preparation, resuspend cells at  $1 \times 10^8$  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).



## 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 CD2 Positive Selection Kit Protocol**

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	 <b>EasySep™</b> (Catalog #18000)	 <b>“The Big Easy”</b> (Catalog #18001)
1	Prepare sample at the indicated cell concentration within the volume range.	1 x 10 <sup>8</sup> cells/mL 0.1 - 2.5 mL NOTE: If starting with fewer than 1 x 10 <sup>7</sup> cells, resuspend cells in 0.1 mL.	1 x 10 <sup>8</sup> cells/mL 0.25 - 8 mL NOTE: If starting with fewer than 2.5 x 10 <sup>7</sup> 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"> <li>• Top up to 5 mL for samples &lt; 1 mL</li> <li>• Top up to 10 mL for samples ≥ 1 mL</li> </ul>
	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 CD2 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 <sup>8</sup> cells/mL 0.25 - 8.5 mL NOTE: If starting with fewer than 2.5 x 10 <sup>7</sup> 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 CD2 Positive Selection 18657HLA	
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

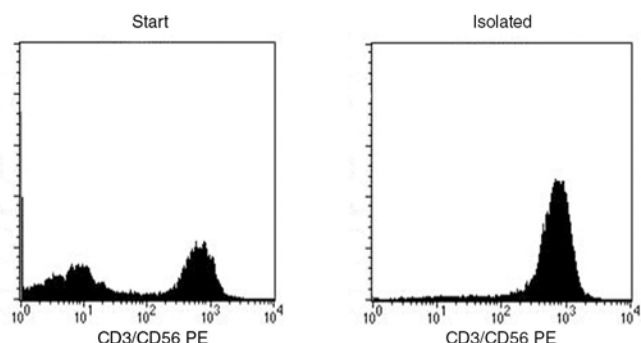
The EasySep™ HLA CD2 Positive Selection Cocktail uses an anti-CD2 antibody clone that to our knowledge blocks all other anti-CD2 antibody clones used to assess purity by flow cytometry. One of the following methods can be used to assess purity:

- Use alternative markers such as fluorochrome-conjugated Anti-Human CD3 Antibody, Clone UCHT1 (Catalog #60011) and Anti-Human CD56 (NCAM) Antibody, Clone HCD56 (Catalog #60021).
- Use a fluorochrome-conjugated secondary antibody, such as Goat Anti-Mouse IgG (H+L) Antibody, Polyclonal (Catalog #60138).

### CD2+ CELL DEPLETION

The EasySep™ HLA CD2 Positive Selection Kit can also be used to deplete CD2+ cells. For further information, contact us at [techsupport@stemcell.com](mailto:techsupport@stemcell.com).

## Data



Starting with PBMCs, the CD2+ cell content of the isolated fraction typically ranges from 86 - 98% (as assessed by labeling with anti-CD3 and anti-CD56 antibodies). In the above example, the purities of the start and final isolated fractions are 51% and 97.7%, respectively.

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