EasySep™

Positive Selection

Catalog #18687HLA

EasySep™ HLA Whole Blood CD2 Positive Selection Kit

For processing 60 mL buffy coat or whole blood

STEMCELL™ TECHNOLOGIES

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Description

Isolate highly purified CD2+ cells from fresh human whole blood by immunomagnetic positive selection.

- Fast and easy-to-use
- Up to 99.6% purity
- No columns required
- Compatible with chimerism testing

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 WB CD2 Positive Selection Cocktail	18687HC	3 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.
EasySep™ Whole Blood Magnetic Particles	18180H	3 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. Do not freeze.	Stable until expiry date (EXP) on label.	A blocking solution required for flow cytometric crossmatch analysis following cell isolation.
EasySep™ Red Blood Cell Lysis Buffer, 10X Concentrate	20110	10 mL	Store at 15 - 25°C. Do not freeze.	Stable until expiry date (EXP) on label.	A 10X concentrated red blood cell lysis reagent.

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.

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
EasySep™ Red Blood Cell Lysis Buffer (1X dilution)	Store at 2 - 8°C. Do not freeze.	Stable for up to 3 months. Do not exceed expiry date (EXP) of original component.

Sample Preparation

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

PERIPHERAL BLOOD

Collect whole blood in a blood collection tube containing anticoagulant.

BUFFY COAT

1. Add an equal volume of recommended medium to whole blood.
2. Centrifuge at 200 x g for 10 minutes at room temperature (15 - 25°C) with the brake off.
3. Remove the concentrated leukocyte band (this is the buffy coat), plus a small portion of the plasma and concentrated red blood cells (RBCs). The target is to concentrate the leukocytes approximately 5-fold while maintaining the same hematocrit.
4. Transfer a maximum of 4.5 mL of buffy coat to the required tube (see Table 1).

Alternatively, HetaSep™ (Catalog #07906) RBC sedimentation can be used to concentrate leukocytes. Please contact Technical Support for further information.

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.

Table 1. EasySep™ HLA Whole Blood CD2 Positive Selection Kit Protocol

		EASYSEP™ MAGNET
STEP	INSTRUCTIONS	**"The Big Easy" (Catalog #18001)** 
1	**Prepare sample within the volume range.**	Up to 4.5 mL
	Add sample to required tube.	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)
2	**Add 1X EasySep™ RBC Lysis Buffer to sample.**	Equal volume to sample
3	**Add Selection Cocktail to sample.**	25 µL/mL of sample
	Mix and incubate.	RT for 15 minutes
4	**Mix Magnetic Particles.**	Pipette up and down more than 5 times
5	**Add Magnetic Particles to sample.**	25 µL/mL of sample
	Mix and incubate.	RT for 10 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 5 mL for samples < 2.5 mL • Top up to 10 mL for samples ≥ 2.5 mL
	Place the tube (without lid) into the magnet and incubate.	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
8	**Repeat steps as indicated.**	Steps 6 and 7, two more times (total of 3 x 5-minute separations)
9	**Resuspend cells in desired medium. Be sure to collect cells from the sides of the tube.**	Isolated cells are ready for use
10	**OPTIONAL: If proceeding to flow cytometric analysis, add Blocking Solution.**	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 Whole Bood CD2 Positive Selection Kit Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #20000 and #21000) 
1	**Prepare sample within the volume range.**	0.25 - 4.5 mL
	Add sample to required tube.	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)
2	**Add 1X EasySep™ RBC Lysis Buffer to sample.**	Equal volume to sample
3	**Select protocol.**	Human CD2 WB Positive Selection 18687HLA
4	**Mix Magnetic Particles.**	Pipette up and down more than 5 times
5	**Load the carousel.**	Follow on-screen prompts
	Start the protocol.	Press the green “Run” button
6	**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
7	**OPTIONAL: If proceeding to flow cytometric analysis, add Blocking Solution.**	200 µL

Notes and Tips

EASYSEP™ RED BLOOD CELL LYSIS BUFFER

EasySep™ Red Blood Cell Lysis Buffer is supplied as a 10X concentrate. Prepare 1X lysis buffer at least 1 hour before use by adding 1 part 10X lysis buffer to 9 parts distilled or Type 1 water. Mix gently and completely before use.

ASSESSING PURITY

The EasySep™ HLA WB CD2 Positive Selection Cocktail uses an anti-CD2 antibody clone that to our knowledge blocks all 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 Antibody, Clone HCD56 (Catalog #60021).
- Use a fluorochrome-conjugated secondary antibody, such as a FITC-labeled sheep anti-mouse IgG.

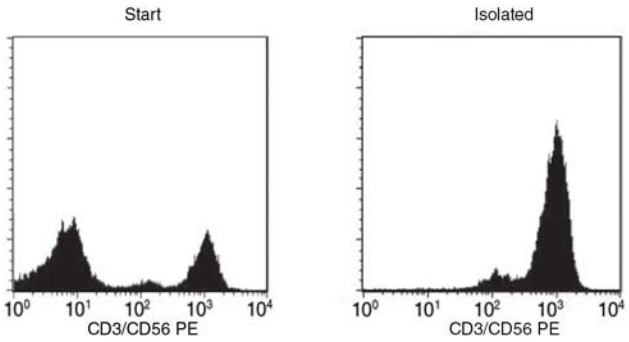
DONOR VARIABILITY

Certain donors express one or more soluble serum factors that can cause cross-linking with magnetic particles. This may result in visible aggregates in the enriched cell fraction following positive selection. These aggregates may appear as a distinct, high side-scatter population on FSC vs. SSC plots during flow cytometry analysis of the enriched fraction. This population consists solely of particles, with no cells or platelets present, as determined by staining with fluorescently-labeled antibodies against dextran, CD41, and CD45.

Potential aggregation can be avoided by washing away the donor plasma. Dilute the sample 2-fold in the recommended medium, and centrifuge at 300 x g for 10 minutes. Remove as much plasma as possible without disturbing the white and red blood cells, then resuspend the sample to the original volume with recommended medium before beginning the separation procedure.

If the samples have not been washed, any aggregates can be gated out during flow cytometry analysis of the enriched fraction based on their FSC vs. SSC characteristics, or by their lack of CD45 expression.

Data



Starting with fresh whole blood, the CD2+ cell content of the isolated fraction (as assessed by labeling with anti-CD3 and anti-CD56) typically ranges from 95.7 - 99.6%. In the above example, the purities of the start and final isolated fractions are 36.2% and 99.2%, respectively.