EasySep™ HLA Total Lymphocyte Enrichment: Complete Processing Kit for Whole Blood

Negative Selection

Catalog #19961HLA

For processing 200 mL whole blood

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Description

Isolate highly purified lymphocytes directly from HetaSep™-treated whole blood or washed leukapheresis samples by immunomagnetic negative selection.

- Fast, easy-to-use and column-free
- Up to 97% purity
- Untouched, viable cells
- Compatible with downstream HLA assays

This kit targets non-lymphocyte cells for removal with antibodies recognizing specific cell surface markers. Unwanted cells are labeled with antibodies and magnetic particles, and separated without columns using an EasySep™ magnet. Desired cells are simply poured off into a new 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 Total Lymphocyte Enrichment Cocktail	19961HC	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.
EasySep™ D Magnetic Particles	19250H	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 TBS.
HetaSep™ 6% Hetastarch Solution	07806	2 x 20 mL	Store at 15 - 25°C.	Stable until expiry date (EXP) on label.	Hetastarch solution used for erythrocyte aggregation.

PBS - phosphate-buffered saline; TBS - TRIS-buffered saline

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.

WHOLE BLOOD

1. Collect whole blood in a blood collection tube containing heparin or ACD as an anticoagulant. Using EDTA as an anticoagulant is not recommended.
2. Based on blood sample volume, select the appropriate size tube according to Table 1. Add 1 part HetaSep™ to 5 parts blood and mix well.
3. a. Place sample at room temperature (15 - 25°C) or 37°C and allow to settle until the red blood cell (RBC) interface is approximately 50% of the total volume.

   OR

   b. Centrifuge the sample according to Table 1 at 90 x g at room temperature (15 - 25°C) with the brake off. Remove sample from centrifuge and allow to sit undisturbed at room temperature for 10 minutes. This will allow further sedimentation of the RBCs and will improve nucleated cell recovery.
4. Harvest the supernatant containing the nucleated cells.
5. Top up the harvested fraction with recommended medium and centrifuge at 120 x g for 10 minutes at room temperature with the brake off.
6. Carefully remove the supernatant.

   OPTIONAL: Lyse remaining RBCs with Ammonium Chloride Solution (Catalog #07800).
7. Resuspend cells in the recommended medium at 1/10th of the original starting volume (e.g. resuspend the cells recovered from 10 mL of whole blood in 1 mL of recommended medium).

Table 1. Sample Preparation Information

START BLOOD VOLUME*	RECOMMENDED TUBE	CENTRIFUGE TIME (MINUTES)	CENTRIFUGE TIME FOR BLOOD ≥ 48 HOURS OLD (MINUTES)
2 mL	5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007)	1	2
3 mL	5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007)	1	4
4 mL	5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007)	2	5
10 mL	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)	5	7
20 mL	50 mL (30 x 115 mm) conical tube (e.g. Catalog #38010)	3 NOTE: For > 24-hour-old blood samples, gravity sedimentation is recommended; see step 3a	Gravity sedimentation recommended; see step 3a
40 mL	50 mL (30 x 115 mm) conical tube (e.g. Catalog #38010)	4 NOTE: For > 24-hour-old blood samples, gravity sedimentation is recommended; see step 3a	Gravity sedimentation recommended; see step 3a

* Start blood volume refers to the volume before HetaSep™ is added.

LEUKAPHERESIS

Cells can be isolated directly from peripheral blood leukapheresis samples without the use of HetaSep™. Instead, wash the leukapheresis sample prior to cell separation as follows:

1. Add 1 part recommended medium to 1 part leukapheresis sample.
2. Centrifuge sample at 140 x g for 10 minutes at room temperature (15 - 25°C) with the brake off.
3. Carefully remove and discard the supernatant.
4. Resuspend cells at 5 x 10^7 cells/mL in recommended medium.

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 pages 1 and 2 for Sample Preparation and Recommended Medium. Refer to Tables 2 and 3 for detailed instructions regarding the EasySep™ procedure for each magnet.

Table 2. EasySep™ HLA Total Lymphocyte Enrichment: Complete Processing Kit for Whole Blood Protocol

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	 **EasySep™ (Catalog #18000)**	**"The Big Easy" (Catalog #18001)** 
1	**Prepare HetaSep™-treated sample within the volume range.**	0.25 - 2 mL	0.25 - 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)
2	**Add Enrichment Cocktail to sample.**	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 10 minutes	RT for 10 minutes
3	**Vortex Magnetic Particles.** **NOTE: Particles should appear evenly dispersed.**	30 seconds	30 seconds
4	**Add Magnetic Particles to sample.**	150 µL/mL of sample	150 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 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	• Top up to 5 mL for samples < 5 mL • Top up to 10 mL for samples ≥ 5 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 the enriched cell suspension into a new tube.**	Isolated cells are immediately compatible with flow cytometric crossmatch analysis and any other downstream application**	Isolated cells are immediately compatible with flow cytometric crossmatch analysis and any other downstream application**

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.
** Residual RBCs can be removed by lysis using Ammonium Chloride Solution if desired.

Table 3. EasySep™ HLA Total Lymphocyte Enrichment: Complete Processing Kit for Whole Blood Protocol

		EASYSEP™ MAGNETS		
STEP	INSTRUCTIONS	EasyEights™ (Catalog #18103)		Easy 50 (Catalog #18002) 
		5 mL tube	14 mL tube	
1	Prepare sample at the indicated cell concentration within the volume range.	0.25 - 2 mL	0.25 - 8 mL	1 - 40 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)	50 mL (30 x 115 mm) conical tube (e.g. Catalog #38010)
2	Add Enrichment Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 10 minutes	RT for 10 minutes	RT for 10 minutes
3	Vortex Magnetic Particles. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds	30 seconds
4	Add Magnetic Particles to sample.	150 µL/mL of sample	150 µL/mL of sample	150 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
5	Add recommended medium to top up 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 < 5 mL • Top up to 10 mL for samples ≥ 5 mL	• Top up to 25 mL for samples ≤ 10 mL • Top up to 50 mL for samples > 10 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 10 minutes	RT for 10 minutes	RT for 5 minutes
6	Carefully pipette** (do not pour) the enriched cell suspension into a new tube.	Isolated cells are immediately compatible with flow cytometric crossmatch analysis and any other downstream application***	Isolated cells are immediately compatible with flow cytometric crossmatch analysis and any other downstream application***	Isolated cells are immediately compatible with flow cytometric crossmatch analysis and any other downstream application***

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]).
*** Residual RBCs can be removed by lysis using Ammonium Chloride Solution if desired.

Directions for Use – Fully Automated RoboSep™ Protocol

See pages 1 and 2 for Sample Preparation and Recommended Medium. Refer to Table 4 for detailed instructions regarding the RoboSep™ procedure.

Table 4. RoboSep™ HLA Total Lymphocyte Enrichment: Complete Processing Kit for Whole Blood Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #20000 and #21000) 
1	Prepare HetaSep™-treated sample within the volume range.	0.25 - 6.5 mL
	Add sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	Select protocol.	Human Total Lymphocyte Negative Selection from WB 19961HLA
3	Vortex Magnetic Particles. NOTE: Particles should appear evenly dispersed.	30 seconds
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.	Isolated cells are immediately compatible with flow cytometric crossmatch analysis and any other downstream application**

** Residual RBCs can be removed by lysis using Ammonium Chloride Solution if desired.

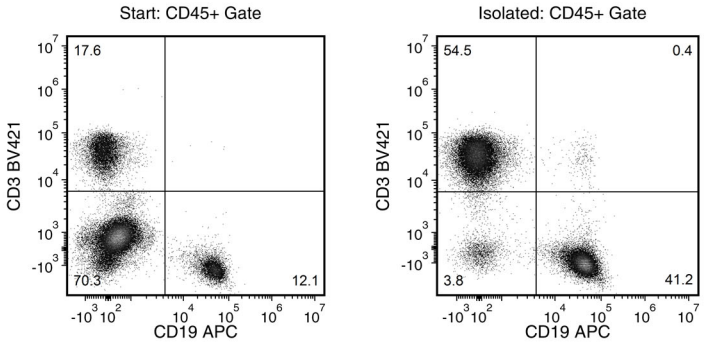
Notes and Tips

ASSESSING PURITY

For purity assessment of lymphocytes (CD3+ and CD19+ cells) by flow cytometry, use the following fluorochrome-conjugated antibody clones:

- Anti-Human CD3 Antibody, Clone UCHT1 (Catalog #60011), and
- Anti-Human CD19 Antibody, Clone HIB19 (Catalog #60005)

Data



Starting with whole blood, the lymphocyte (CD3+ and CD19+) content of the enriched fraction is typically 96.8 ± 0.5% (mean SD using the purple EasySep™ Magnet). In the above example, the purities of the start and final enriched fractions are 29.7% and 95.7%, respectively.