RosetteSep™ Human ILC2 Enrichment Kit

Catalog #15382

For processing 200 mL whole blood

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Description

Enrich untouched group 2 innate lymphoid cells (ILC2s) directly from human whole blood by negative selection.

- Fast and easy-to-use
- Requires no special equipment or training
- Untouched, viable cells
- Can be combined with SepMate™ for consistent, high-throughput sample processing
- Facilitates rapid flow sorting of ILC2s

This kit targets non-ILC2s for removal with antibodies recognizing specific cell surface markers.The RosetteSep™ antibody cocktail crosslinks unwanted cells in human whole blood to multiple red blood cells (RBCs), forming immunorosettes. This increases the density of the unwanted (rosetted) cells, such that they pellet along with the free RBCs when centrifuged over a density gradient medium. Desired cells are never labeled with antibody and are easily collected as an enriched population at the interface between the plasma and the density gradient medium. Enriched cells are immediately available for downstream applications such as flow cytometry or cell sorting.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
RosetteSep™ Human ILC2 Enrichment Cocktail	15382C	5 x 2 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A combination of monoclonal antibodies in PBS.

PBS - phosphate-buffered saline

Components may be shipped at room temperature (15 - 25°C) but should be stored as indicated above.

Sample Preparation

For available whole blood products, see www.stemcell.com/primarycells.

For optimal performance, use whole peripheral blood collected within the last 24 hours and stored at room temperature (15 - 25°C).

Although RosetteSep™ has been optimized for use with whole blood, cells can be enriched from other sources (i.e. buffy coat) provided that RBCs are present at a ratio of at least 100 RBCs per nucleated cell. The concentration of nucleated cells in the sample should not exceed 5 x 10^7 cells/mL.

For more rapid RosetteSep™ processing, this product can be combined with the SepMate™ RUO (Catalog #86450/86415) or SepMate™ IVD* (Catalog #85450/85415) cell isolation tube. For more information on SepMate™, see the associated Product Information Sheets (PIS).

If using SepMate™ with samples with hematocrits outside the normal range, please note that a minimum packed RBC volume is required. See table below for details.

	SEPMATE™-15	SEPMATE™-50
Sample volume range	0.5 - 5 mL	4 - 17 mL
Minimum packed RBC volume	0.25 mL	2 mL
Maximum packed RBC volume	3 mL	12 mL

- For samples with low hematocrits, the required sample volume may be greater than the minimum volume stated above.
- For samples with very high hematocrits, the maximum sample volume may be less than the maximum volume stated above.

* 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

Dulbecco's Phosphate Buffered Saline with 2% Fetal Bovine Serum (Catalog #07905).

Density Gradient Medium

Lymphoprep™ (Catalog #07801) or other density gradient medium with a density of 1.077 g/mL.

Directions for Use – RosetteSep™ Protocol

See page 1 for Sample Preparation and Recommended Medium.

Ensure that whole blood sample, recommended medium, density gradient medium, and centrifuge are all at room temperature (15 - 25°C). For more information on the use of the SepMate™-15 or SepMate™-50 tube, refer to the applicable PIS.

Table 1. RosetteSep™ Human ILC2 Enrichment Kit Protocol

		ROSETTESEP™	
STEP	INSTRUCTIONS	Standard Tube	SepMate™ Tube
1	Collect sample.	Up to 15 mL per tube (see Table 2)	0.5 - 17 mL per tube (see Table 2)
2	Add RosetteSep™ 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	Dilute sample with recommended medium and mix gently.	Equal volume to sample	Equal volume to sample
4	Add density gradient medium to required tube.	See Table 2 for volumes and tubes	See Table 2 for volumes and tubes
5	Add diluted sample to the tube containing the density gradient medium.	Layer diluted sample on density gradient medium, being careful to minimize their mixing	Pour or pipette diluted sample into tube
6	Centrifuge.	800 x g for 20 minutes, **brake off**	1200 x g for 10 minutes, **brake on** NOTE: For samples > 24-hours old it may be necessary to centrifuge for an additional 10 minutes.
7	Collect enriched cells. * For platelet removal see footnote below.	Harvest enriched cell layer with a pipette and transfer to new tube**	Pour supernatant into a new standard tube NOTE: Some RBCs may be present on the surface of the SepMate™ insert after centrifugation. This will not affect performance.
8	Wash enriched cells.	Top up with recommended medium	Top up with recommended medium
9	Centrifuge.	300 x g for 10 minutes brake low	300 x g for 10 minutes brake low
		Discard supernatant	Discard supernatant
10	Repeat steps as indicated.	Steps 8 and 9***	Steps 8 and 9***
11	Resuspend cells in recommended medium.	The enriched cells are ready for use	The enriched cells are ready for use

RT - room temperature (15 - 25°C)
* To minimize platelet contamination, remove and discard the top third of the plasma layer before collecting the cells at the density gradient medium:plasma interface. Platelets may also be removed by including an extra wash with centrifugation at 120 x g for 10 minutes at room temperature with no brake after step 9.
** Sometimes it is difficult to see the cells at the interface. It is recommended to remove some of the density gradient medium along with the pre-enriched cells in order to ensure complete recovery.
*** One of the wash steps can be done with Ammonium Chloride Solution (Catalog #07800) prior to flow cytometry analysis or if residual RBCs will interfere with subsequent assays.

Table 2. Recommended Volumes and Tube Sizes

WHOLE BLOOD VOLUME	RECOMMENDED MEDIUM VOLUME	STANDARD TUBE		SEPMATE™ TUBE	
		TUBE SIZE	DENSITY GRADIENT MEDIUM VOLUME	TUBE SIZE	DENSITY GRADIENT MEDIUM VOLUME*
0.5 mL	0.5 mL	5 mL	1.5 mL	15 mL	4.5 mL
1 mL	1 mL	5 mL	1.5 mL	15 mL	4.5 mL
2 mL	2 mL	14 mL	3 mL	15 mL	4.5 mL
3 mL	3 mL	14 mL	3 mL	15 mL	4.5 mL
4 mL	4 mL	14 mL	4 mL	15 mL / 50 mL	4.5 mL** / 15 mL
5 mL	5 mL	50 mL	15 mL	15 mL / 50 mL	3.5 mL / 15 mL
10 mL	10 mL	50 mL	15 mL	50 mL	15 mL
15 mL	15 mL	50 mL	15 mL	50 mL	15 mL
17 mL	17 mL	--	--	50 mL	15 mL

* Small bubbles may be present in the density gradient medium after pipetting. This will not affect performance.
** If using a sample size of > 4 - 5 mL in the SepMate™-15 tube, use 3.5 mL of density gradient medium.

Notes and Tips

CONVERSION OF g TO RPM

To convert g to RPM, use the following formula:

$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5}) \times (Radius)}}$$

Where: RCF = relative centrifugal force (g)
RPM = centrifuge speed in revolutions per minute
Radius = radius of rotor in cm

ASSESSING PURITY

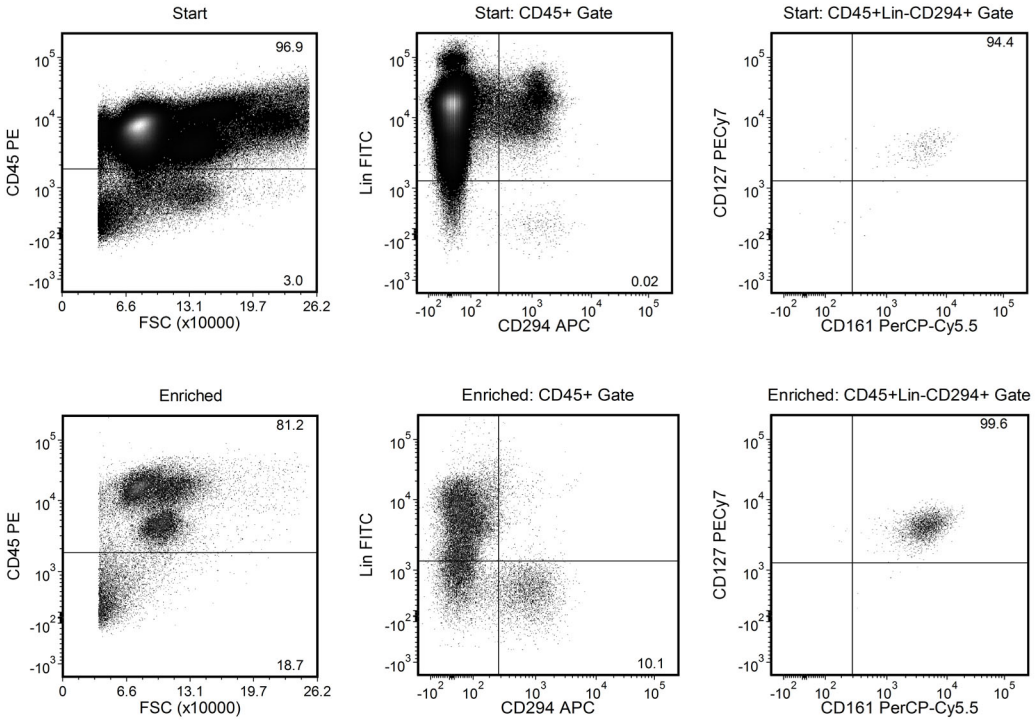
ILC2s are described as CD45-positive, lineage-negative, CD127-positive, CD161-positive, and CD294-positive. For purity assessment of ILC2s by flow cytometry use the following fluorochrome-conjugated antibodies:

- Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018), and
- Anti-human CD127 (IL-7Ra) antibody, clone A019D5, and
- Anti-human CD161 (KLRB1) antibody, clone HP-3G10, and
- Anti-human CD294 (CRTH2) antibody, clone BM16, and
- Anti-human lineage-specific antibodies (see below)

For lineage-specific antigen labeling use the following fluorochrome-conjugated antibodies:

- Anti-human CD1a antibody, clone HI149, and
- Anti-Human CD3 Antibody, Clone UCHT1 (Catalog #60011), and
- Anti-human CD4 antibody, clone RPA-T4, and
- Anti-human CD11c antibody, clone 3.9, and
- Anti-Human CD14 Antibody, Clone M5E2 (Catalog #60004), and
- Anti-Human CD16 Antibody, Clone 3G8 (Catalog #60041), and
- Anti-Human CD19 Antibody, Clone HIB19 (Catalog #60005), and
- Anti-Human CD34 Antibody, Clone 581 (Catalog #60013), and
- Anti-human CD94 antibody, clone DX22, and
- Anti-Human CD123 (IL-3Ra) Antibody, Clone 6H6 (Catalog #60110), and
- Anti-human CD303 antibody, clone 201A, and
- Anti-human FceR1a antibody, clone AER-37, and
- Anti-human TCR alpha/beta antibody, clone IP26, and
- Anti-human TCR gamma/delta antibody, clone B1

Data



Starting with fresh whole blood, the ILC2 content (CD45+Lin-CD294+CD127+CD161+) of the enriched fraction typically ranges from 0.44 - 53%. In the above example, the percentage of ILC2s in the start and final enriched fractions are 0.02% and 10.1%, respectively.

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