EasySep™ Human ILC2 Enrichment Kit

Negative Selection

Catalog #17972

For processing 1 x 10^9 cells

STEMCELL™ TECHNOLOGIES

Scientists Helping Scientists™ | WWW.STEMCELL.COM

TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713

INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM

FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

Document #DX20788 | Version 1_1_1

Description

Enrich untouched Group 2 Innate Lymphoid Cells (ILC2s) from washed leukapheresis samples by immunomagnetic negative selection.

- Fast, easy-to-use and column-free
- Untouched, viable cells
- Facilitates rapid flow sorting of ILC2s

This kit targets non-ILC2s 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 and cell sorting.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Human ILC2 Enrichment Cocktail	17972C	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™ Dextran RapidSpheres™ 50103	50103	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.

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

LEUKAPHERESIS

Wash the peripheral blood leukapheresis sample by adding an equivalent volume of recommended medium or PBS containing 2% fetal bovine serum (FBS). Centrifuge at 300 x g for 10 minutes at room temperature (15 - 25°C). If red blood cell (RBC) lysis is desired, lyse with Ammonium Chloride Solution (Catalog #07800). If platelet removal is desired, centrifuge at 120 x g for 10 minutes with the brake off. Remove the supernatant and resuspend the cells at 1 x 10^8 cells/mL in recommended medium.

Recommended Medium

EasySep™ Buffer (Catalog #20144) or PBS containing 2% 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 Tables 1 and 2 for detailed instructions regarding the EasySep™ procedure for each magnet.

Table 1. EasySep™ Human ILC2 Enrichment Kit Protocol

		EASYSEP™ MAGNET
STEP	INSTRUCTIONS	**"The Big Easy" (Catalog #18001)** 
1	**Prepare sample within the volume range.**	1 x 10^8 cells/mL 1 - 5 mL
	Add sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	**Add Enrichment Cocktail to sample.**	100 µL/mL of sample
	Mix and incubate.	RT for 10 minutes
3	**Vortex Magnetic Particles.** **NOTE: Particles should appear evenly dispersed.**	1 minute
4	**Add Magnetic Particles to sample.**	100 µL/mL of sample
	Mix and incubate.	RT for 1 minute
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 5 mL for samples ≤ 2 mL • Top up to 10 mL for samples > 2 mL
	Place the tube (without lid) into the magnet and incubate.	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.**	Use a new 14 mL tube
7	**Remove the tube from the magnet and place the new tube (without lid) into the magnet and incubate for a second separation.**	RT for 5 minutes
8	**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 ready for use

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.

Table 2. EasySep™ Human ILC2 Enrichment Kit Protocol

		EASYSEP™ MAGNET
STEP	INSTRUCTIONS	**Easy 50 (Catalog #18002)** 
1	Prepare sample at the indicated cell concentration within the volume range.	1 x 10^8 cells/mL 5 - 25 mL
	Add sample to required tube.	50 mL (30 x 115 mm) conical tube (e.g. Catalog #38010)
2	Add Enrichment Cocktail to sample.	100 µL/mL of sample
	Mix and incubate.	RT for 10 minutes
3	Vortex Magnetic Particles. NOTE: Particles should appear evenly dispersed.	1 minute
4	Add Magnetic Particles to sample.	100 µL/mL of sample
	Mix and incubate.	RT for 1 minute
5	Add recommended medium to top up sample to the indicated volume. Vortex	• Top up to 22 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 15 minutes
6	Carefully pipette** (do not pour) off the enriched cell suspension into a new tube.	Use a new 50 mL tube
7	Remove the tube from the magnet and place the new tube (without lid) into the magnet and incubate for a second separation.	RT for 15 minutes
8	Carefully pipette** (do not pour) off the enriched cell suspension into a new tube.	Isolated cells are ready for use

RT - room temperature (15 - 25°C)
** Collect the entire supernatant, all at once, into a single pipette.

Notes and Tips

ASSESSING PURITY

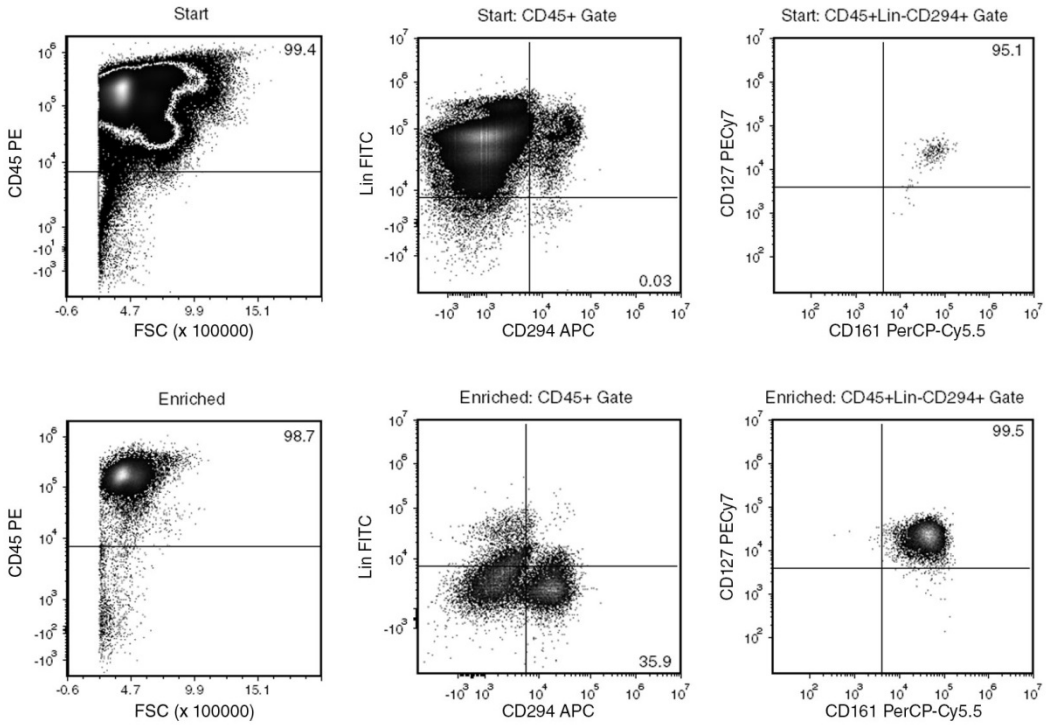
ILC2s are described as lineage-negative, CD45-positive, CD127-positive, CD161-positive, and CD294-positive. For purity assessment of ILC2s by flow cytometry, use the following fluorochrome-conjugated antibodies:

- 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 PBMCs, the ILC2 content (Lin-CD45+CD294+CD127+CD161+) of the enriched fraction typically ranges from 13 - 78%. In the above example, the percentages of ILC2s in the start and final enriched fractions are 0.03% and 35.3%, respectively.

NOTE: The ILC2 content of the start fraction typically ranges from 0.001 - 0.16%.