EasySep™ Human ILC2 Isolation Kit

Positive Selection

Catalog #17782

For processing 2 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 #DX21876 | Version 1_0_0

Description

Isolate highly purified group 2 innate lymphoid cells (ILC2s) from fresh peripheral blood mononuclear cells (PBMCs) or washed leukapheresis samples by immunomagnetic positive selection.

- Easy-to-use and column-free
- Up to 95% purity
- Facilitates rapid isolation of ILC2s without flow sorting
- No-wash removal of EasySep™ Releasable RapidSpheres™

First, CRTH2+ cells are isolated by column-free immunomagnetic positive selection using antibody complexes and EasySep™ Releasable RapidSpheres™. Then, bound magnetic particles are removed from the EasySep™-isolated CRTH2+ cells, and unwanted non-ILC2s are targeted for depletion using antibody complexes and EasySep™ Dextran RapidSpheres™. The final isolated fraction contains highly purified ILC2s that are immediately ready for downstream applications. Following cell isolation with this EasySep™ kit, antibody complexes remain bound to the cell surface and may interact with Brilliant Violet™ antibody conjugates, polyethylene glycol-modified proteins, or other chemically related ligands.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Human CRTH2-PE Positive Selection Cocktail	17782CA	1 x 0.1 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A monoclonal antibody in PBS, 0.1% BSA, and 0.05% sodium azide.
EasySep™ Release PE Positive Selection Cocktail	17654C	2 x 0.5 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A combination of monoclonal antibodies in PBS and 0.1% BSA.
EasySep™ Human ILC2 Isolation Cocktail	17782CB	1 x 0.5 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A combination of monoclonal antibodies in PBS.
EasySep™ Releasable RapidSpheres™ 50201	50201	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™ 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.
EasySep™ Anti-Human CD32 (Fc gamma RII) Blocker for Positive Selection	18520	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™ Release Buffer	20145	1 x 1 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A buffer for release of Releasable RapidSpheres™ from cells following positive selection.

BSA - bovine serum albumin; 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.

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. After preparation, resuspend cells at 2 x 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).

LEUKAPHERESIS (LEUKO PAK)

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 2 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 Table 1 for detailed instructions regarding the EasySep™ procedure for each magnet.

Table 1. EasySep™ Human ILC2 Isolation Kit Protocol

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	 **EasySep™ (Catalog #18000)**	**"The Big Easy" (Catalog #18001)** 
1	**Prepare sample at the indicated cell concentration within the volume range.**	2 x 10^8 cells/mL 1 mL NOTE: If starting with fewer than 2 x 10^8 cells, resuspend cells in 1 mL.	2 x 10^8 cells/mL 1 - 4 mL NOTE: If starting with fewer than 2 x 10^8 cells, resuspend cells in 1 mL.
2	**Add FcR blocker to sample.**	100 µL/mL of sample	100 µL/mL of sample
3	**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)
4	**Add CRTH2-PE Positive Selection Cocktail to sample.**	10 µL/mL of sample	10 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 and centrifuge. Resuspend sample in original volume.**	Top up to 4 mL with recommended medium and centrifuge. Carefully aspirate and discard supernatant. Resuspend in the same volume as step 1.	Top up to 12 mL with recommended medium and centrifuge. Carefully aspirate and discard supernatant. Resuspend in the same volume as step 1.
6	**Add Release PE Positive Selection Cocktail to sample.**	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
7	**Vortex Releasable RapidSpheres™.** **NOTE: Particles should appear evenly dispersed.**	30 seconds	30 seconds
8	**Add Releasable RapidSpheres™ to sample.**	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
9	**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 ≤ 1 mL • Top up to 10 mL for samples > 1 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 15 minutes	RT for 15 minutes
10	**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
11	**Repeat steps as indicated.**	Steps 9 and 10 (total of 2 x 15-minute separations)	Steps 9 and 10 (total of 2 x 15-minute separations)
Continue to step 12, next page		Continue to step 12, next page	Continue to step 12, next page

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS (CONTINUED)	 EasySep™ (Catalog #18000)	"The Big Easy" (Catalog #18001) 
12	Add recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times. Be sure to collect cells off the sides of the tube.	Top up to 1 mL	Top up to 1 mL
13	Add Release Buffer to sample.	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
14	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
	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes
15	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 5 mL tube	Use a new 14 mL tube
16	Centrifuge.	Carefully aspirate and discard supernatant. Resuspend in 1 mL.	Carefully aspirate and discard supernatant. Resuspend in 1 mL.
17	Add ILC2 Isolation Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 10 minutes	RT for 10 minutes
18	Vortex Dextran RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
	Add Dextran RapidSpheres™ to sample and mix.	50 µL/mL of sample NO INCUBATION, proceed immediately to next step	50 µL/mL of sample NO INCUBATION, proceed immediately to next step
19	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
	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes
20	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 5 mL tube	Use a new 14 mL tube
21	Add Dextran RapidSpheres™ to the new tube containing the enriched cells and mix.	50 µL NO INCUBATION, proceed immediately to next step	50 µL NO INCUBATION, proceed immediately to next step
22	Place the tube from step 21 (without lid) into the magnet and incubate for a second separation.	RT for 5 minutes**	RT for 5 minutes**
23	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	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.

** Purity may be improved by performing an additional 5-minute magnetic separation.

Notes and Tips

ASSESSING PURITY

ILC2s are described as CD45-positive, lineage-negative, CD127-positive, CD161-positive, and CD294 (CRTH2)-positive. For purity assessment of ILC2s by flow cytometry use the following fluorochrome-conjugated antibodies:

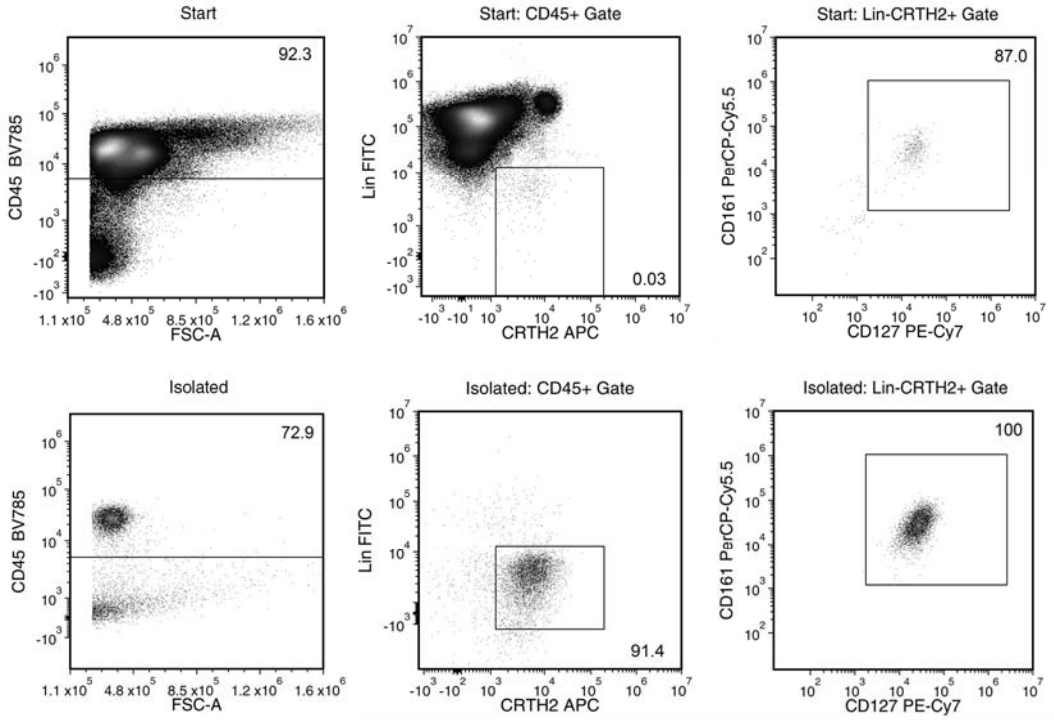
- Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018), and
- Anti-human CD127 (IL-7Ra) antibody, clone A019D5, and
- Anti-human CD161 (KLRB1) antibody, clone HP-3G10, and
- Anti-human CD294 (CRTH2) antibody, clone BM16, and
- Anti-human lineage-specific antibodies (see below)

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 (CRA-1), and
- Anti-human TCR alpha/beta antibody, clone IP26, and
- Anti-human TCR gamma/delta antibody, clone B1

NOTE: Brilliant Violet™ antibody conjugates should be carefully titrated on EasySep™ Release-isolated cells prior to analysis by flow cytometry or fluorescence microscopy. For purity assessment with Brilliant Violet™ antibody conjugates, use of BD Horizon Brilliant™ Stain Buffer is recommended to reduce non-specific interactions. For more information, refer to the manufacturer's instructions or contact us at techsupport@stemcell.com.

Data



Starting with fresh leukapheresis samples, the ILC2 content (CD45+Lin- CRTH2+CD161+CD127+) of the isolated fraction typically ranges from 84 - 95%. In the above example, the purities of the start and final isolated fractions are 0.03% and 91% (gated on CD45), respectively.