EasySep™ Human Gamma/Delta T Cell Isolation Kit

For processing 1 x 10^9 cells

Catalog #19255

Catalog #19255RF RoboSep™

Negative Selection

Document #10000000882 | Version 02

STEMCELL™ TECHNOLOGIES

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Description

Isolate untouched and highly purified gamma/delta T cells from fresh or previously frozen human peripheral blood mononuclear cells (PBMCs) or lysed leukapheresis samples by immunomagnetic negative selection.

- Fast, easy-to-use, and column-free
- Up to 97% purity
- Isolated cells are untouched

This kit targets non-gamma/delta T 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.

If using culture-expanded PBMCs, contact techsupport@stemcell.com for more information.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Human Gamma/Delta T Cell Isolation Cocktail	19255C	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™ D2 Magnetic Particles	19650	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.

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.

PERIPHERAL BLOOD

Prepare a PBMC suspension from whole peripheral 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.

If using previously frozen PBMCs, incubate the cells with DNase I Solution (Catalog #07900) at a concentration of 100 µg/mL at room temperature (15 - 25°C) for at least 15 minutes prior to labeling and separation. Filter aggregated suspensions through a 37 µm cell strainer (Catalog #27215) for optimal results.

After preparation, resuspend cells at 5 x 10^7 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).

LYSED LEUKAPHERESIS

1. Add 4 parts Ammonium Chloride Solution (Catalog #07800) to 1 part leukapheresis sample.

   NOTE: If working with large volumes (> 20 mL), concentrate the Leukopak first by centrifuging at 300 x g for 10 minutes. Remove the supernatant and resuspend the cells in 1/10th of the original Leukopak volume with recommended medium (e.g. for 30 mL of cells, resuspend in 3 mL of recommended medium and add 12 mL of Ammonium Chloride Solution). For small volumes (≤ 20 mL), add Ammonium Chloride Solution directly to the Leukopak.
2. Incubate on ice for 15 minutes.
3. Wash the cells by topping up the tube with recommended medium. Centrifuge at 300 x g for 10 minutes at room temperature (15 - 25°C). Remove the supernatant.
4. OPTIONAL (FOR PLATELET REMOVAL):

   a. Wash the cells by topping up the tube with recommended medium. Centrifuge the cells at 120 x g for 10 minutes at room temperature with the brake off. Carefully remove the supernatant.

   b. Repeat step 4a one or more times until most of the platelets have been removed (indicated by a clear supernatant).
5. Resuspend the 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 1 and 2 for detailed instructions regarding the EasySep™ procedure for each magnet.

Table 1. EasySep™ Human Gamma/Delta T Cell 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.	5 x 10^7 cells/mL 0.5 - 2 mL	5 x 10^7 cells/mL 0.5 - 8.5 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 Isolation Cocktail to sample. NOTE: Do not vortex cocktail.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 15 minutes	RT for 15 minutes
3	Vortex Magnetic Particles. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
4	Add Magnetic Particles to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 10 minutes	RT for 10 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 ≤ 4 mL • Top up to 10 mL for samples > 4 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.	Use a new 5 mL tube	Use a new 14 mL tube
7	Vortex Magnetic Particles. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
8	Add Magnetic Particles to the enriched cell suspension in the new tube.	37.5 µL	• 37.5 µL for samples ≤ 4 mL • 75 µL for samples > 4 mL
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
9	Remove the original tube from the magnet; place the new tube (without lid) containing the enriched cells into the magnet and incubate for a second separation.	RT for 5 minutes	RT for 5 minutes
10	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.

Table 2. EasySep™ Human Gamma/Delta T Cell Isolation Kit Protocol

		EASYSEP™ MAGNETS
STEP	INSTRUCTIONS	**Easy 50 (Catalog #18002)** 
1	Prepare sample at the indicated cell concentration within the volume range.	5 x 10^7 cells/mL 1 - 40 mL
	Add sample to required tube.	50 mL (30 x 115 mm) conical tube (e.g. Catalog #38010)
2	Add Isolation Cocktail to sample. NOTE: Do not vortex cocktail.	50 µL/mL of sample
	Mix and incubate.	RT for 15 minutes
3	Vortex Magnetic Particles. NOTE: Particles should appear evenly dispersed.	30 seconds
4	Add Magnetic Particles to sample.	50 µL/mL of sample
	Mix and incubate.	RT for 10 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 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
6	Carefully pipette* (do not pour) the enriched cell suspension into a new tube.	Use a new 50 mL tube
7	Vortex Magnetic Particles. NOTE: Particles should appear evenly dispersed.	30 seconds
8	Add Magnetic Particles to the enriched cell suspension in the new tube.	• 200 µL for samples ≤ 10 mL • 400 µL for samples > 10 mL
	Mix and incubate.	RT for 5 minutes
9	Remove the original tube from the magnet; place the new tube (without lid) containing the enriched cells into the magnet and incubate for a second separation.	RT for 10 minutes
10	Carefully pipette* (do not pour) 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.

Directions for Use – Fully Automated RoboSep™ Protocol

See pages 1 and 2 for Sample Preparation and Recommended Medium. Refer to Table 3 for detailed instructions regarding the RoboSep™ procedure.

Table 3. RoboSep™ Human Gamma/Delta T Cell Isolation Kit Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #21000) 
1	Prepare sample at the indicated cell concentration within the volume range.	5 x 10^7 cells/mL 0.5 - 8.5 mL
	Add sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	Select protocol.	• For sample volumes ≤ 4 mL: Human Gamma-Delta T Cell Negative Selection 19255-small volume • For sample volumes > 4 mL: Human Gamma-Delta T Cell Negative Selection 19255-large volume
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 ready for use

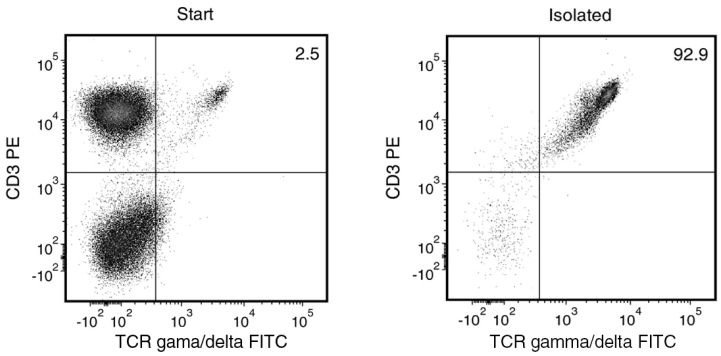
Notes and Tips

ASSESSING PURITY

For purity assessment of gamma/delta T cells by flow cytometry, use the following fluorochrome-conjugated antibody clones:

- Anti-Human CD3 Antibody, Clone UCHT1 (Catalog #60011), and
- Anti-human TCR gamma/delta antibody (e.g. clone IMMU510 or 11F2)

Data



Starting with fresh PBMCs, the gamma/delta T cell content (TCR gamma/delta+CD3+) of the isolated fraction typically ranges from 90 - 97%. In the above example, the purities of the start and final isolated fractions are 2.5% and 92.9%, respectively.