EasySep™ Human Pan-NK Cell Isolation Kit

For processing 1 x 10^9 cells

Catalog #100-1580
#100-1662 RoboSep™

Negative Selection

Document #10000028771 | Version 00

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Description

Isolate untouched and highly purified pan-NK cells from fresh or previously frozen human peripheral blood mononuclear cells (PBMCs) or washed leukapheresis samples in as little as 8 minutes by immunomagnetic negative selection. This kit has been designed without using anti-human CD36 or HLA-DR antibodies, allowing for improved retention of NK cell subsets.

- Fast, easy-to-use, and column-free
- Up to 96% purity with high recovery
- Retain CD36+ and HLA-DR+ NK cells
- Isolated cells are untouched

This kit targets non-NK 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 or pipetted 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™ Human Pan-NK Cell Isolation Cocktail	300-1051	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	2 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 to 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.

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 #27250) 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).

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). Remove the supernatant and 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% 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 Pan-NK 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 0.25 - 2 mL	5 x 10^7 cells/mL 0.5 - 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 Isolation Cocktail to sample. NOTE: Do not vortex cocktail.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
3	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
4	Add RapidSpheres™ to sample and mix.	50 µL/mL of sample	50 µL/mL of sample
	No incubation needed	No incubation, IMMEDIATELY move to next step	No incubation, IMMEDIATELY move to next step
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 3 minutes	RT for 3 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 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 Pan-NK Cell Isolation Kit Protocol

		EASYSEP™ MAGNETS		
		EasyEights™ (Catalog #18103)		
STEP	INSTRUCTIONS	5 mL tube	14 mL tube	Easy 50 (Catalog #18002)
1	Prepare sample at the indicated cell concentration within the volume range.	5 x 10^7 cells/mL 0.25 - 2 mL	5 x 10^7 cells/mL 0.5 - 8 mL	5 x 10^7 cells/mL 4 - 45 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 Isolation Cocktail to sample. NOTE: Do not vortex cocktail.	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
3	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds	30 seconds
4	Add RapidSpheres™ to sample and mix.	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample
	No incubation needed.	No incubation, IMMEDIATELY move to next step	No incubation, IMMEDIATELY move to the next step	No incubation, IMMEDIATELY move to the next step
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 ≤ 4 mL • Top up to 10 mL for samples > 4 mL	• Top up to 25 mL for samples ≤ 20 mL • Top up to 50 mL for samples > 20 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 10 minutes
6	Carefully pipette** (do not pour) the enriched cell suspension into a new tube.	Use a new 5 mL tube	Use a new 14 mL tube	Use a new 50 mL tube
7	Add RapidSpheres™ to the new tube containing the enriched cells and mix.	Use same volume as in step 4 No incubation, IMMEDIATELY move to next step	Use same volume as in step 4 No incubation, IMMEDIATELY move to next step	Use same volume as in step 4 No incubation, IMMEDIATELY move to next step
8	Remove the tube from the magnet; place the new tube (without lid) into the magnet and incubate for a second separation.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
9	Carefully pipette** (do not pour) the enriched cell suspension into a new tube.	Isolated cells are ready for use	Isolated cells are ready for use	Isolated cells are ready for use

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]).

Directions for Use – Fully Automated RoboSep™ Protocol

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

Table 3. RoboSep™ Human Pan-NK 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 mL
	Add sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	Select protocol.	Human Pan-NK Cell Isolation Kit – 100-1580
3	Vortex RapidSpheres™. 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 NK cells (CD3-CD56+) by flow cytometry, use the following fluorochrome-conjugated antibody clones:

- Anti-Human CD3 Antibody, Clone UCHT1 (Catalog #60011), and
- Anti-Human CD56 Antibody, Clone HCD56 (Catalog #60021), and
- Anti-Human CD36 Antibody, Clone FA6-152 (Catalog #60084), and
- Anti-Human HLA-DR Antibody, Clone L243 (Catalog #100-0312), and
- Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018; optional)

Data

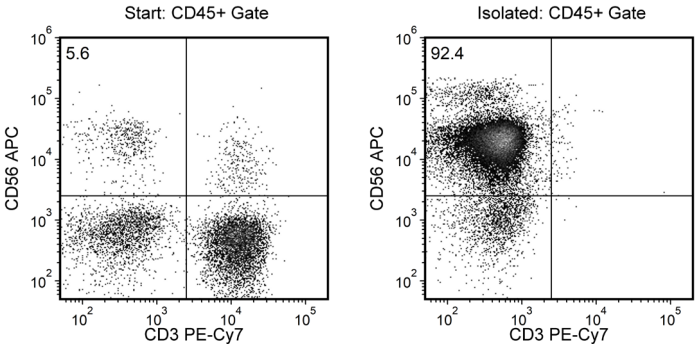

Figure 1. Typical Cell Expression Profile after EasySep™ Human Pan-NK Cell Isolation

Starting with human PBMCs from healthy donors, the NK cell (CD3-CD56+) content of the isolated fraction is typically 89.4 ± 6.6% (mean ± SD). In the above example, the purities of the start and final isolated fractions are 5.6% and 92.4%, respectively.

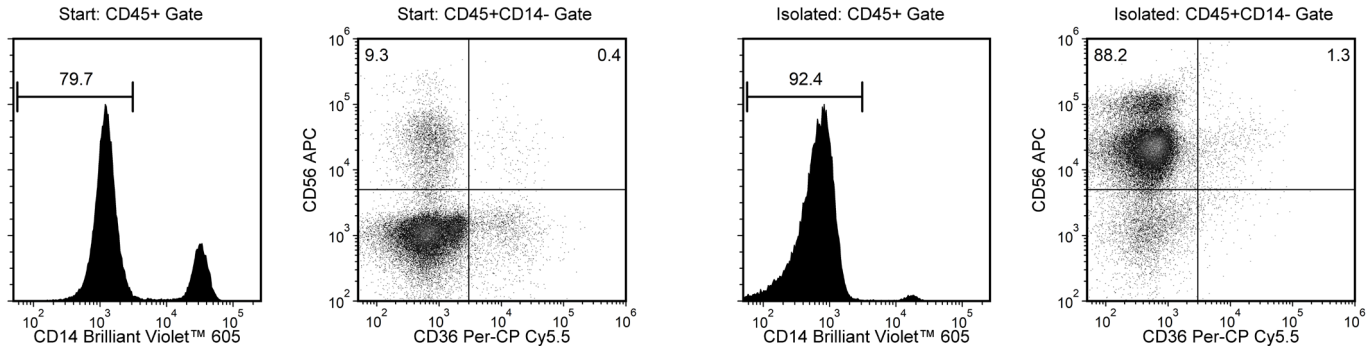

Figure 2. EasySep™ Human Pan-NK Cell Isolation Profile of a Sample from a Donor with a High Body Mass Index (BMI)

Starting with human PBMCs obtained from a donor with a BMI > 30, the CD36+CD56+ NK cell content of the start and isolated fractions were 0.3% and 1.2%, respectively. CD36 is also expressed on non-NK cell subsets, including CD14+ cells, which were gated out to accurately measure NK cell content.
Note: NK cells obtained from donors with a high BMI may have a higher proportion of CD36+ NK cells.

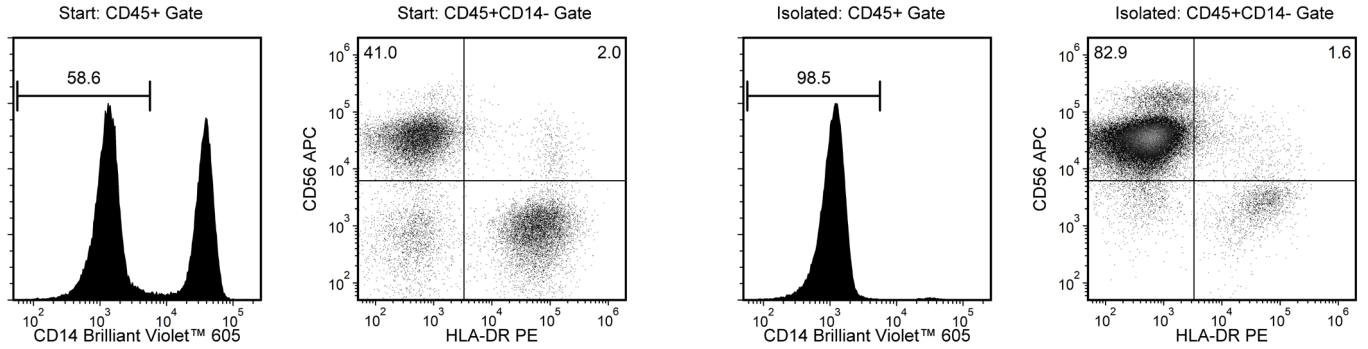

Figure 3. EasySep™ Human Pan-NK Cell Isolation Profile of a Sample from a Patient with Systemic Lupus Erythematosus (SLE)

Starting with human PBMCs obtained from a patient with SLE, the HLA-DR+CD56+ NK cell content of the start and isolated fractions were 1.2% and 1.6%, respectively. HLA-DR is also expressed on non-NK cell subsets, including CD14+ cells, which were gated out to accurately measure the NK cell content.
Note: NK cells obtained from donors with SLE may have a higher proportion of HLA-DR+ NK cells.