EasySep™ Human Neutrophil Isolation Kit

Negative Selection

Catalog #17957

For processing 1 x 10^9 cells

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Description

Isolate untouched and highly purified neutrophils from fresh human peripheral blood leukocytes by immunomagnetic negative selection.

- Fast, easy-to-use and column-free
- Up to 99% purity
- Untouched, viable cells

This kit targets non-neutrophils 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.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Human Neutrophil Isolation Cocktail	17957C	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

Important: Do not use dextran sedimentation to prepare cells.

WHOLE BLOOD USING RED BLOOD CELL (RBC) LYSIS (preferred for slightly higher purity)

1. Collect whole blood in a blood collection tube containing anticoagulant.
2. Carefully perform a standard density gradient separation (e.g. using Lymphoprep™; Catalog #07801). Do not use SepMate™.
3. Remove and discard the plasma layer, the band of mononuclear cells and the density gradient medium, leaving the RBC pellet intact.
4. Add 9 parts Ammonium Chloride Solution (Catalog #07800) to 1 part RBC pellet and mix well.
5. Incubate on ice for 15 minutes. Centrifuge at 500 x g for 10 minutes with the brake on low.
6. Discard supernatant and wash pellet with cold (2 - 8°C) recommended medium, centrifuging at 120 x g for 10 minutes with the brake off.
7. Discard supernatant and resuspend cells at 5 x 10^7 cells/mL in cold recommended medium.

WHOLE BLOOD USING HETASEP™ RBC SEDIMENTATION (preferred for faster, lysis-free sample processing)

1. Collect whole blood in a blood collection tube containing anticoagulant.
2. Add 1 part HetaSep™ (Catalog #07906) to 5 parts whole blood and mix well. Use the minimum-sized tube for the total volume of HetaSep™:blood sample. A 14 mL tube is the maximum size recommended for optimal leukocyte recovery.
3. Centrifuge sample at 110 x g for 6 minutes at room temperature (15 - 25°C) with the brake off.
4. Remove tube from centrifuge and let sit undisturbed (maximum 15 minutes) until the RBC:plasma interface is approximately 40% of the total volume.
5. Harvest leukocyte-rich plasma (everything above the RBC fraction) into a 50 mL tube and add 4 parts cold (2 - 8°C) recommended medium to 1 part harvested cells/plasma.
6. Centrifuge at 500 x g for 10 minutes at room temperature with the brake on low.
7. Discard supernatant and wash pellet with cold recommended medium to remove excess platelets, centrifuging at 120 x g for 10 minutes at room temperature with the brake off.
8. Discard supernatant and resuspend cells at 5 x 10^7 cells/mL in cold 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 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 Neutrophil 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 0.25 - 6.5 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.**	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	2 - 8°C for 5 minutes	2 - 8°C for 5 minutes
3	**Vortex RapidSpheres™.** **NOTE: Particles should appear evenly dispersed.**	30 seconds	30 seconds
4	**Add RapidSpheres™ to sample.**	40 µL/mL of sample	40 µL/mL of sample
	Mix and incubate.	2 - 8°C for 3 minutes	2 - 8°C for 3 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 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.**	Use a new 5 mL 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.**	RT for 3 minutes	RT for 3 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	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 Neutrophil Isolation Kit Protocol

		EASYSEP™ MAGNETS		
STEP	INSTRUCTIONS	 EasyPlate™ (Catalog #18102)	 EasyEights™ (Catalog #18103) 5 mL tube	 EasyEights™ (Catalog #18103) 14 mL tube
1	Prepare sample at the indicated cell concentration within the volume range.	5 x 10^7 cells/mL 0.05 - 0.2 mL	5 x 10^7 cells/mL 0.25 - 2 mL	5 x 10^7 cells/mL 0.5 - 6.5 mL
	Add sample to required tube (or plate if using the EasyPlate™ EasySep™ Magnet).	Round-bottom, non-tissue culture-treated 96-well plate (e.g. Catalog #38018)	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.	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	2 - 8°C for 5 minutes	2 - 8°C for 5 minutes	2 - 8°C for 5 minutes
3	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds	30 seconds
4	Add RapidSpheres™ to sample.	40 µL/mL of sample	40 µL/mL of sample	40 µL/mL of sample
	Mix and incubate.	2 - 8°C for 3 minutes	2 - 8°C for 3 minutes	2 - 8°C for 3 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 0.25 mL	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 or plate (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
6	Carefully pipette (do not pour) the enriched cell suspension** into a new tube or plate.	Use a new well in the 96-well plate	Use a new 5 mL tube	Use a new 14 mL tube
7	Remove the tube or plate, containing the isolated cells, from the magnet and place the new tube or plate (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
8	Carefully pipette (do not pour) the enriched cell suspension** into a new tube or plate.	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 Neutrophil Isolation Kit Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #20000 and #21000) 
1	Prepare sample at the indicated cell concentration within the volume range.	5 x 10^7 cells/mL 0.5 - 6.5 mL
	Add sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	Select protocol.	Human Neutrophil Negative Selection 17957-high purity
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

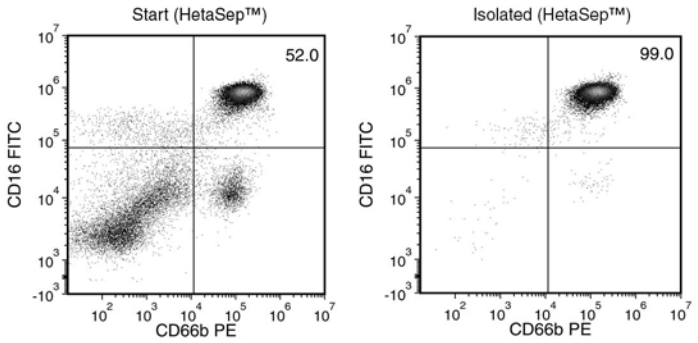
ASSESSING PURITY

For purity assessment of neutrophils (CD16+CD66b+) by flow cytometry use the following fluorochrome-conjugated antibody clones:

- Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018), and
- Anti-Human CD16 Antibody, Clone 3G8 (Catalog #60041), and
- Anti-Human CD66b Antibody, Clone G10F5 (Catalog #60086)

Alternatively, purity may be assessed by performing a cytospin on the enriched cells followed by Wright’s or May-Grünwald staining (e.g. Sigma Catalog #W0625 or #MG500, respectively).

Data



Starting with whole blood prepared using HetaSep™ or Lymphoprep™ with RBC lysis, the neutrophil content (CD45+CD16+CD66b+) of the isolated fraction typically ranges from 98.7 ± 0.9% (mean ± SD). In the above example, the purities of the start and final isolated fractions are 52.0% and 99.0%, respectively.