EasySep™ Human Pan-Granulocyte Isolation Kit

Negative Selection

Catalog #19259

For processing 1 x 10^9 cells

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Description

Isolate highly purified pan-granulocytes (neutrophils, eosinophils, and basophils) 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-granulocytes 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 Pan-Granulocyte Isolation Cocktail	19259C	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	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 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

Important: Do not use dextran sedimentation to prepare cells.

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

1. Collect whole blood in a blood collection tube containing anticoagulant.
2. Add 4 parts Ammonium Chloride Solution (Catalog #07800) to 1 part whole blood and mix well.
3. Incubate on ice for 15 minutes then centrifuge at 300 x g for 10 minutes.
4. Wash pellet once with recommended medium and centrifuge at 120 x g for 10 minutes with the brake off.
5. Discard supernatant and resuspend cells at 5 x 10^7 cells/mL in 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 90 x g for 2 minutes (if total volume ≤ 5 mL) or 5 minutes (if total volume > 5 mL) at room temperature (15 - 25°C) with the brake off.
4. Remove tube from centrifuge and let sit undisturbed for 10 minutes.
5. Harvest the leukocyte-rich plasma (everything above the RBC fraction) into a 50 mL tube and add 4 parts recommended medium to 1 part harvested cells/plasma.
6. Centrifuge at 300 x g for 10 minutes at room temperature.
7. Discard supernatant and wash pellet 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 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 Pan-Granulocyte 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.**	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 10 minutes	RT for 10 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 5 minutes	RT for 5 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.**	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-Granulocyte 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.	50 µL/mL of sample
	Mix and incubate.	RT for 10 minutes
3	Vortex Magnetic Particles. NOTE: Particles should appear evenly dispersed.	30 seconds
4	Add Magnetic Particles to sample.	75 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 10 mL for samples < 5 mL • Top up to 25 mL for samples ≥ 5 - 15 mL • Top up to 50 mL for samples > 15 - 40 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	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	Carefully pipette** (do not pour) the enriched cell suspension into a new tube.	Isolated cells are ready for use

** Collect the entire supernatant, all at once, into a single pipette (e.g. for volumes of 5 - 10 mL use a 10 mL serological pipette [Catalog #38004], for volumes > 10 - 25 mL use a 25 mL serological pipette [Catalog #38005], and for volumes > 25 mL use a 50 mL serological pipette [Catalog #38006]).

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-Granulocyte 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 - 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-Granulocyte Isolation 19259
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

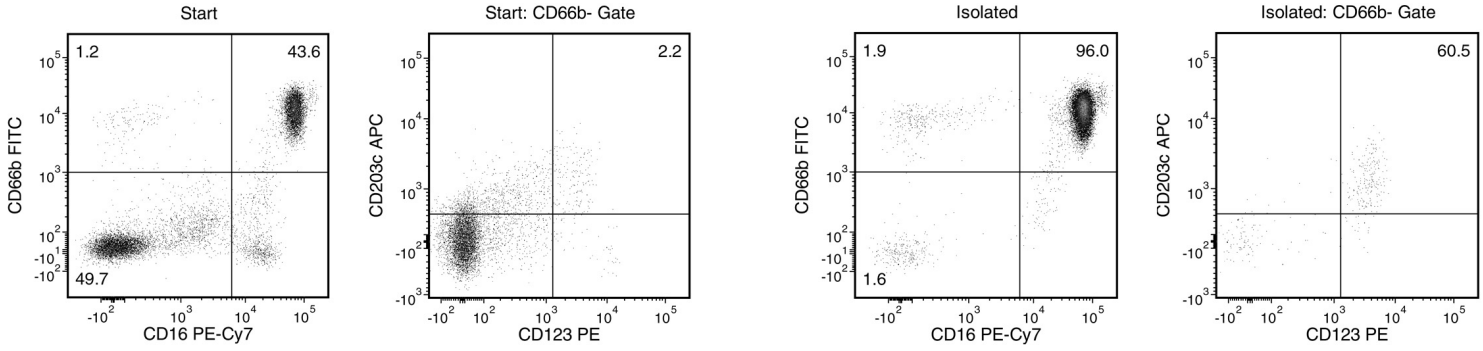
For purity assessment of pan-granulocytes (neutrophils, eosinophils, and basophils) by flow cytometry, use the following fluorochrome-conjugated antibody clones:

- Anti-Human CD16 Antibody, Clone 3G8 (Catalog #60041), and
- Anti-Human CD66b Antibody, Clone G10F5 (Catalog #60086), and
- Anti-Human CD123 Antibody (IL-3Rα), Clone 6H6 (Catalog #60110; optional, for basophil assessment), and
- Anti-human IgE antibody (optional, for basophil assessment), and
- Anti-human CD203c antibody (optional, for basophil assessment)

Neutrophils are CD66b+CD16+, eosinophils are CD66b+CD16- and low in forward scatter but high in side scatter, and basophils are CD66-CD123+IgE+ or CD66-CD123+CD203c^{low}.

Alternatively, purity may be assessed by performing a cytospin of the enriched cells followed by Wright’s or May-Grünwald staining (e.g. Sigma-Aldrich Catalog #W0625 or #205435, respectively).

Data



Starting with whole peripheral blood, the total granulocyte content of the isolated fraction typically ranges from 97 - 99%. In the above example, neutrophils are typically CD66b+CD16+, eosinophils are typically CD66b+CD16-, and basophils are CD66b- and can be further defined as CD203c+CD123+ and IgE+ (not shown).