



EasySep™ Human Pan-Granulocyte Isolation Kit

Negative Selection

Catalog #19259

For processing 1×10^9 cells



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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

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Description

Isolate highly purified pan-granulocytes (neutrophils, eosinophils, and basophils) from fresh human peripheral blood mononuclear cells 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 $300 \times g$ for 10 minutes.
4. Wash pellet once with recommended medium and centrifuge at $120 \times g$ for 10 minutes with the brake off.
5. Discard supernatant and resuspend cells at 5×10^7 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 $90 \times g$ 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 $300 \times g$ for 10 minutes at room temperature.
7. Discard supernatant and wash pellet to remove excess platelets, centrifuging at $120 \times g$ for 10 minutes at room temperature with the brake off.
8. Discard supernatant and resuspend cells at 5×10^7 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. Corning Catalog #352058)	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)
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.	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 off the enriched cell suspension into a new tube.	Isolated cells are now ready for use	Isolated cells are now 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.	1 x 10 ⁸ cells/mL 1 - 40 mL	
	Add sample to required tube.	50 mL conical tube (e.g. Corning Catalog #352070)	
2	Add Isolation Cocktail to sample.	50 µL/mL of sample	
	Mix and incubate.	RT for 10 minutes	
3	Vortex Magnetic Particles.	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 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.	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, for volumes > 10 - 25 mL use a 25 mL serological pipette and for volumes > 25 mL use a 50 mL serological pipette).

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 Enrichment 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 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)	
2	Select protocol.	Human Pan-Granulocyte Isolation 19259	
3	Vortex Magnetic Particles.	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

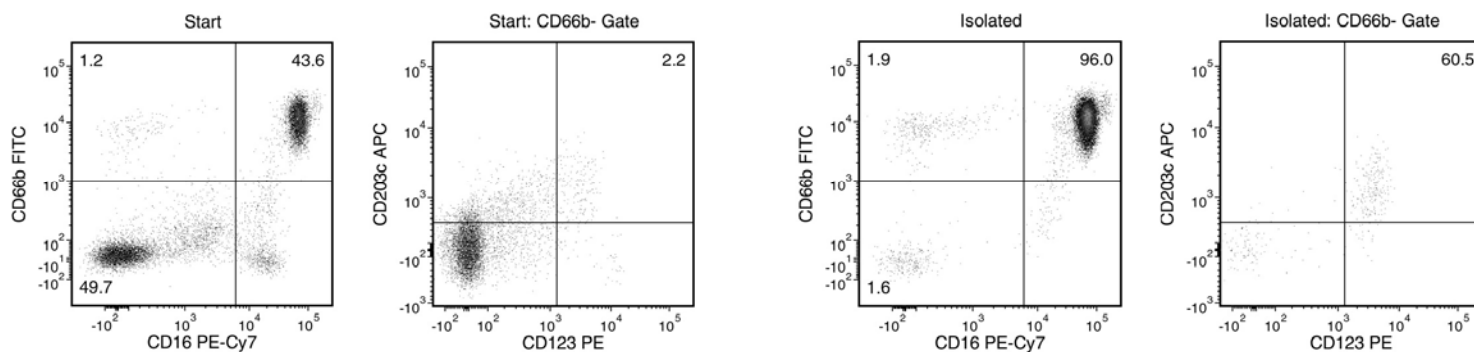
For purity assessment of pan-granulocytes (neutrophils, eosinophils, and basophils) by flow cytometry use the following fluorochrome-conjugated antibody clones:

- Anti-Human CD16 Antibody, Clone 3G8 (Catalog #60041), and
- Anti-Human CD66b Antibody, Clone G10F5 (Catalog #60086), and
- Anti-Human CD123 Antibody (IL-3R α), 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%. As shown 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).

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