EasySep™ Human Eosinophil Enrichment Kit

Negative Selection

Catalog #19256

For processing 1 x 10^9 cells

STEMCELL™ TECHNOLOGIES

Scientists Helping Scientists™ | WWW.STEMCELL.COM

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

Document #29039 | Version 1_0_3

Description

Isolate untouched and highly purified eosinophils (CD16-CD66b+CD45+) from fresh human peripheral blood polymorphonuclear cells (PMNCs) immunomagnetic negative selection.

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

This kit targets non-eosinophils 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 Eosinophil Enrichment Cocktail	19256C	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™ Magnetic Nanoparticles	19150.1	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 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 Ammonium Chloride Solution (Catalog #07800) to the RBC pellet and mix well.
5. Incubate on ice for 10 minutes then centrifuge at 300 x g for 8 minutes.
6. Discard supernatant and wash pellet with cold recommended medium, centrifuging at 250 x g for 10 minutes.
7. 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 50 x g for 5 minutes at room temperature (15 - 25°C) with the brake off.
4. Remove tube from centrifuge and let sit undisturbed (maximum 10 minutes) until the RBC : plasma interface is approximately 40% of the total volume.
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 500 x g for 10 minutes at room temperature with the brake on low.
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 Table 1 for detailed instructions regarding the EasySep™ procedure for each magnet.

Table 1. EasySep™ Human Eosinophil Enrichment 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 1 - 2 mL	5 x 10^7 cells/mL 1 - 6.5 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 Selection Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	2 - 8°C for 10 minutes	2 - 8°C for 10 minutes
3	Mix Magnetic Particles. NOTE: Particles should appear evenly dispersed.	Pipette up and down more than 5 times	Pipette up and down more than 5 times
4	Add Magnetic Particles to sample.	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	2 - 8°C for 10 minutes	2 - 8°C 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 < 2 mL • Top up to 10 mL for samples ≥ 2 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 10 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 Isolated cells are ready for use	Use a new 14 mL tube Isolated cells are ready for use
OPTIONAL ADDITIONAL SEPARATION NOTE: This will improve purity but may reduce recovery.		---	---
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	RT for 10 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.

Directions for Use – Fully Automated RoboSep™ Protocol

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

Table 2. RoboSep™ Human Eosinophil 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 1 - 6.5 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 Eosinophil Negative Selection 19256-high purity
3	**Mix Magnetic Particles.** **NOTE: Particles should appear evenly dispersed.**	Pipette up and down more than 5 times
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

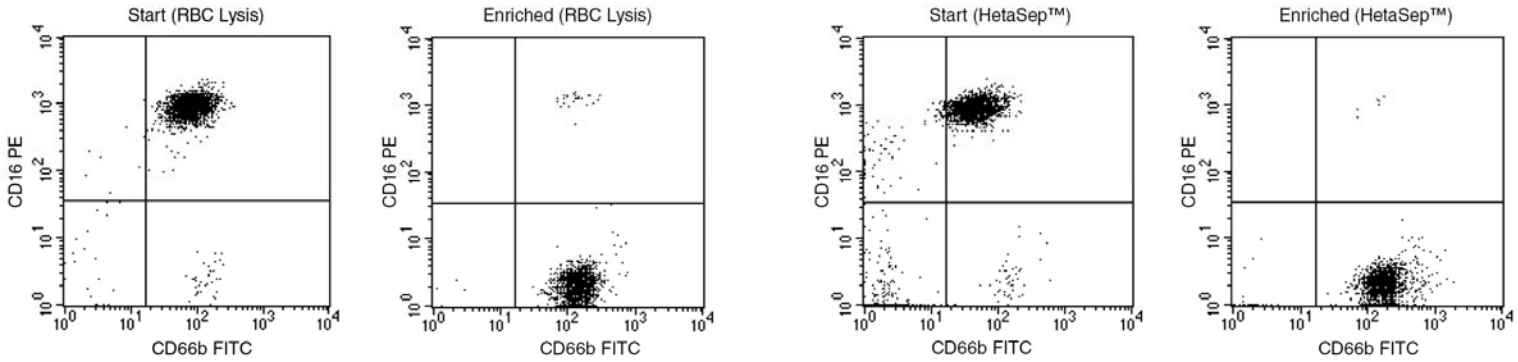
For purity assessment of eosinophils by flow cytometry use the following fluorochrome-conjugated antibody clones:

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

Eosinophils are CD16-CD66b+ and are low in forward scatter but high in side scatter.

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

Data



Starting with whole blood prepared using RBC lysis or HetaSep™, the eosinophil content (CD16-CD66b+CD45+) of the enriched fraction typically ranges from 86 - 99%. In the above examples, the purities of the start and final enriched fractions prepared using RBC lysis are 1.2% and 98% (gated on CD45+), respectively and prepared using HetaSep™ are 1.2% and 96% (gated on CD45+), respectively.