EasySep™ Human Eosinophil Isolation Kit

For processing 1 x 10^9 cells

Catalog #17956
Catalog #17956RF RoboSep™

Negative Selection

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Description

Isolate untouched and highly purified eosinophils (CD16-CD66b+CD45+) from fresh human peripheral blood polymorphonuclear cells (PMNCs) by 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 Isolation Cocktail	17956C	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 then centrifuge at 300 x g for 8 minutes.
6. Discard supernatant and wash pellet with cold (2 - 8°C) recommended medium, centrifuging at 250 x g for 10 minutes.
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 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 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 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 Eosinophil 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 1 - 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.** **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 No incubation, IMMEDIATELY proceed to next step	50 µL/mL of sample No incubation, IMMEDIATELY proceed 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.**	Use a new 5 mL tube	Use a new 14 mL tube
7	**Remove the tube from the magnet; place the new tube (without lid) into the magnet and incubate for a second separation.**	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 Eosinophil Isolation Kit Protocol

		EASYSEP™ MAGNETS	
		EasyEights™ (Catalog #18103)	
STEP	INSTRUCTIONS	5 mL tube	14 mL tube
1	Prepare sample at the indicated cell concentration within the volume range.	5 x 10^7 cells/mL 0.5 - 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. 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 No incubation, IMMEDIATELY proceed to next step	50 µL/mL of sample No incubation, IMMEDIATELY proceed 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 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
7	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 10 minutes
8	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

RT - room temperature (15 - 25°C)
** Collect the entire supernatant, all at once, into a single pipette (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 Eosinophil 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 1 - 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 Eosinophil Negative Selection 17956-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

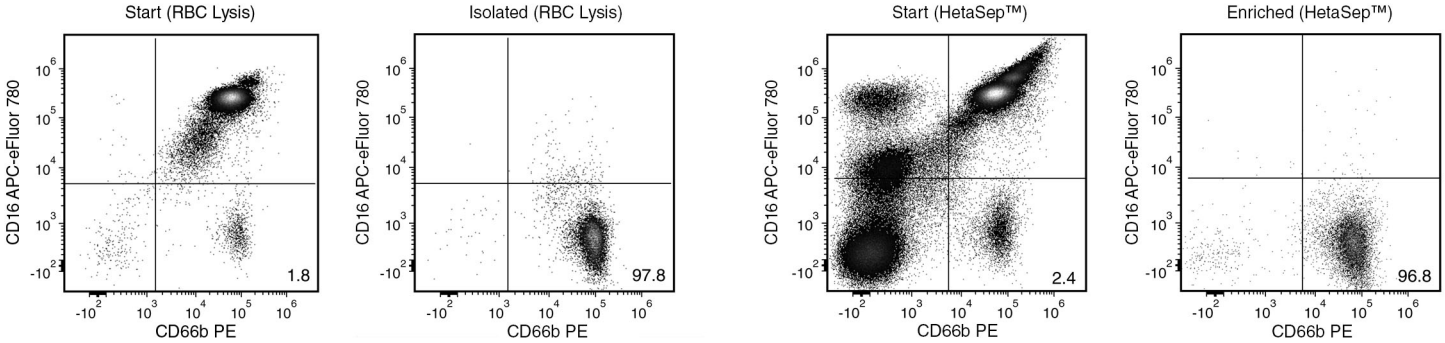
For purity assessment of eosinophils by flow cytometry, use the following fluorochrome-conjugated antibody clones:

- 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)

NOTE: 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 Catalog #W0625 or #205435, respectively).

Data



Starting with whole blood prepared using RBC lysis or HetaSep™, the eosinophil content (CD16-CD66b+CD45+) of the isolated fraction is typically 96.5 ± 2.5% (mean ± SD using the purple EasySep™ Magnet). In the above examples, the purities of the start and final isolated fractions prepared using RBC lysis are 1.8% and 97.8% (gated on CD45+), respectively, and when prepared using HetaSep™, the purities are 2.4% and 96.8% (gated on CD45+), respectively.