

EasySep™ Direct Human Eosinophil Isolation Kit

Negative Selection
Catalog #19656

For processing 100 mL whole blood



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Description

Isolate highly purified eosinophils directly from human whole blood by immunomagnetic negative selection.

The benefits of this kit include:

- Effectively depletes RBCs without the need for density gradient centrifugation, sedimentation, or lysis
- Up to 98% purity of isolated cells
- Fast, easy-to-use and column-free
- Isolated cells are untouched

This kit targets non-eosinophils for removal with antibodies recognizing specific cell surface markers. Unwanted cells are labeled with antibodies and EasySep™ Direct RapidSpheres™, and separated using an EasySep™ magnet. Desired cells are simply collected into a new tube and 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™ Direct Human Eosinophil Isolation Cocktail	19656C	2 x 2.5 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A combination of monoclonal antibodies in PBS. Includes an Fc receptor blocking antibody.
EasySep™ Direct RapidSpheres™ 50300	50300	4 x 2.5 mL	Store at 2 - 8°C. Do not freeze	Stable until expiry date (EXP) on label.	A suspension of magnetic particles and monoclonal antibodies in PBS.

PBS - phosphate-buffered saline

Components may be shipped at room temperature (15 - 25°C) and should be refrigerated upon receipt.

Precipitate may be observed in the cocktail vial but will not affect performance.

Sample Preparation

PERIPHERAL BLOOD

The presence of EDTA is important for the performance of this kit. Collect blood using K2EDTA or K3EDTA as an anticoagulant. If an anticoagulant other than EDTA is used, EDTA must be added to the whole blood sample to a final concentration of 3 mM.

For best recovery, use unprocessed human whole blood. Recovery of the desired isolated cells decreases with samples that are older than 24 hours.

The volume of blood that can be processed depends on the EasySep™ magnet used for the isolation procedure. Blood samples must be placed in the required tube to properly fit into the appropriate EasySep™ magnet (see Tables 1 and 2).


Recommended Medium

PBS containing 1 mM EDTA. Medium should be free of Ca⁺⁺ and Mg⁺⁺. EasySep™ Buffer (Catalog #20144) may also be used.

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™ Direct Human Eosinophil Isolation Kit Protocol



		EASYSEP™ MAGNET
STEP	INSTRUCTIONS	“The Big Easy” (Catalog #18001) 
1	Collect sample within the volume range.	2 - 7 mL
	Add whole blood sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds
3	Add Isolation Cocktail to sample.	50 µL/mL of sample
4	Add RapidSpheres™ to sample.	50 µL/mL of sample
5	Mix and incubate.	RT for 5 minutes
6	Add recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	<ul style="list-style-type: none"> • Top up to 10 mL for samples < 4 mL • Top up to 12 mL for samples ≥ 4 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes
7	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 14 mL tube
8	Add RapidSpheres™ to the new tube containing the enriched cells.	25 µL/mL of original sample volume
	Mix and incubate.	RT for 5 minutes
9	Remove the tube from the magnet and place the new tube from step 8 (without lid) into the magnet and incubate for a second separation.	RT for 5 minutes
10	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 14 mL tube
11	Add RapidSpheres™ to the new tube containing the enriched cells.	25 µL/mL of original sample volume
	Mix and incubate.	RT for 5 minutes
12	Remove the tube from the magnet and place the new tube from step 11 (without lid) into the magnet and incubate for a third separation.	RT for 5 minutes
13	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

RT - room temperature (15 - 25°C)

* Following the first magnetic separation the collected cells may contain a significant amount of RBCs and may look similar to the original unprocessed human whole blood sample.

** To minimize RBC contamination in the isolated cells, pour off the sample along a clean area of the tube (i.e. the opposite side to where the sample was poured in).

Table 2. EasySep™ Direct Human Eosinophil Isolation Kit Protocol

STEP	INSTRUCTIONS	EASYSEP™ MAGNETS	
		 EasyEights™ (Catalog #18103) 14 mL tube	 Easy 50 (Catalog #18002)
1	Collect sample within the volume range.	3 - 6 mL	7 - 25 mL
	Add whole blood sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)	50 mL (30 x 115 mm) conical tube (e.g. Catalog #38010)
2	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
3	Add Isolation Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample
4	Add RapidSpheres™ to sample.	50 µL/mL of sample	50 µL/mL of sample
5	Mix and incubate.	RT for 5 minutes	RT for 5 minute
6	Add recommended medium to top up sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	<ul style="list-style-type: none"> • Top up to 10 mL for samples < 4 mL • Top up to 12 mL for samples ≥ 4 mL 	Top up to double the original sample volume
	Place the tube (without lid) into the magnet and incubate.	RT for 10 minutes	RT for 10 minutes
7	Carefully pipette*** (do not pour) the enriched cell suspension into a new tube. NOTE: Collect the entire clear fraction from top to bottom. For optimal recovery, also collect a small volume of RBCs (up to 10% of the starting sample volume).	Use a new 14 mL tube	Use a new 50 mL tube
8	Add RapidSpheres™ to the new tube containing the enriched cells.	25 µL/mL of original sample volume	25 µL/mL of original sample volume
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
9	Remove the tube from the magnet and place the new tube from step 8 (without lid) into the magnet and incubate for a second separation.	RT for 5 minutes	RT for 5 minutes
10	Carefully pipette*** (do not pour) the enriched cell suspension into a new tube. NOTE: Collect only the clear fraction.	Use a new 14 mL tube	Use a new 50 mL tube
11	Add RapidSpheres™ to the new tube containing the enriched cells.	25 µL/mL of original sample volume	25 µL/mL of original sample volume
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
12	Remove the tube from the magnet and place the new tube from step 11 (without lid) into the magnet and incubate for a third separation.	RT for 5 minutes	RT for 5 minutes
13	Carefully pipette*** (do not pour) the enriched cell suspension into a new tube. NOTE: Collect only the clear fraction.	Isolated cells are ready for use	Isolated cells are ready for use

RT - room temperature (15 - 25°C)

*** Collect the entire enriched cell suspension, all at once, into a single pipette (e.g. for EasyEights™ 5 mL tube use a 5 mL serological pipette [Catalog #38002]; for EasyEights™ 14 mL tube use a 10 mL serological pipette [Catalog #38004]).

Notes and Tips

REMOVAL OF RESIDUAL RBCs IN ISOLATED CELLS

Typically, further RBC depletion is not required following cell isolation. If residual RBCs are visible in the isolated cell pellet following centrifugation after the end of the protocol, resuspend in a small volume (0.2 - 2.5 mL) of recommended medium or desired culture medium and place in a smaller EasySep™ magnet for an additional 5-minute separation. Collect the supernatant; the isolated cells are ready for use in downstream applications. Residual RBCs may also be lysed using Ammonium Chloride Solution (Catalog #07800).

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), and
- Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018; optional)

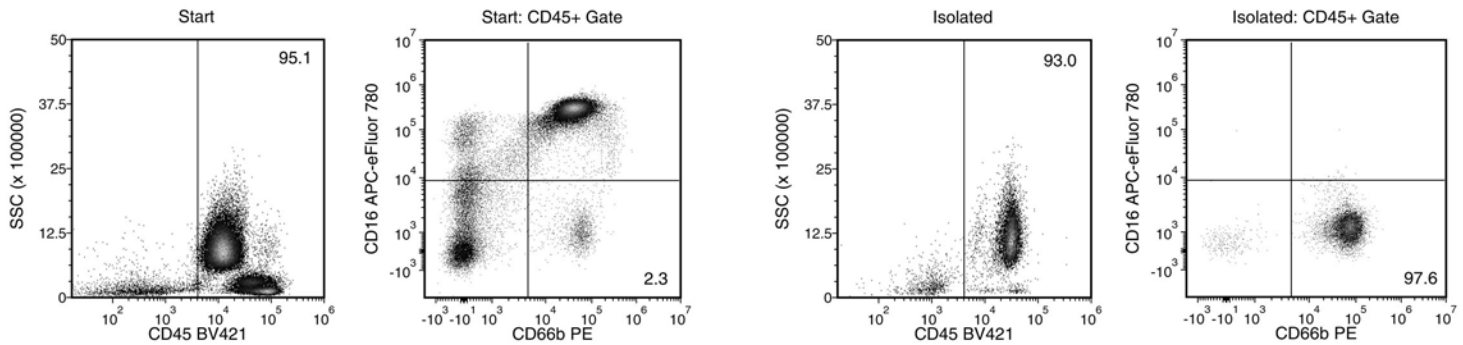
NOTE: Eosinophils are CD16-CD66b+ and are low in forward scatter but high in side scatter.

NOTE: It is recommended to assess purity on the CD45+ cells to exclude debris, platelets, and RBCs. Include a viability dye if necessary (e.g. Propidium Iodide [Catalog #75002]; 7-AAD [7- Aminoactinomycin D; Catalog #75001]).

Alternatively, purity may be assessed by performing a cyto-spin on the enriched cells followed by Wright's or May-Grünwald staining (e.g. Sigma Catalog #W0625 or #205435, respectively).

Data

Starting with human whole blood from normal healthy donors, the typical eosinophil (CD16-CD66b+) content of the non-lysed final isolated fraction is $95.8 \pm 2.7\%$ (gated on CD45).



In the above example, the eosinophil content of the lysed whole blood start sample and the non-lysed final isolated fraction is 2.3% and 97.6% (gated on CD45), respectively, or 2.2% and 90.8% (not gated on CD45), respectively.

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