

EasySep™ Human Basophil Isolation Kit

Negative Selection
Catalog #17969

For processing 1 x 10⁹ cells



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Description

Isolate untouched and highly purified basophils from HetaSep™-processed human peripheral blood by immunomagnetic negative selection.

- Fast, easy-to-use and column-free
- Up to 98% purity
- Isolated cells are untouched

This kit targets non-basophils 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 Basophil Isolation Cocktail	17969C	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	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 water.

PBS - phosphate-buffered saline

Components may be shipped at room temperature (15 - 25°C) but should be stored as indicated above.

Sample Preparation

For available fresh and frozen samples, see www.stemcell.com/primarycells.

PERIPHERAL BLOOD

IMPORTANT: Do not use dextran sedimentation to prepare cells.

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.
NOTE: 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 110 x g for 6 minutes at room temperature (15 - 25°C) with the brake off.
4. Remove tube from centrifuge and let sit undisturbed until red blood cell (RBC):plasma interface is at approximately 40% of the total volume (a maximum of 10 minutes).
5. Harvest the plasma containing the nucleated cells (everything above the RBC fraction) into a 50 mL tube, and add 4 parts cold recommended medium to 1 part harvested cells/plasma.
6. Centrifuge at 500 x g for 10 minutes at room temperature with the brake set to 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 10⁷ 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 Basophil 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 - 1.5 mL	5 x 10 ⁷ cells/mL 0.5 - 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.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 7 minutes	RT for 7 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	<ul style="list-style-type: none"> • 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 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.	Use a new 5 mL tube	Use a new 14 mL tube
7	Remove the tube from the magnet and place the new tube (without lid) into the magnet and incubate.	RT for 3 minutes	RT for 5 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 Basophil Isolation Kit Protocol

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	EasyEights™ (Catalog #18103)	
		5 mL tube	14 mL tube
1	Prepare sample at the indicated cell concentration within the volume range.	5 x 10 ⁷ cells/mL 0.25 - 1.5 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.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 7 minutes	RT for 7 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	<ul style="list-style-type: none"> • 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 10 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 and place the new tube (without lid) into the magnet and incubate for a second round of separation.	RT for 10 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 Basophil 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 - 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 Basophil Negative Selection 17969-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

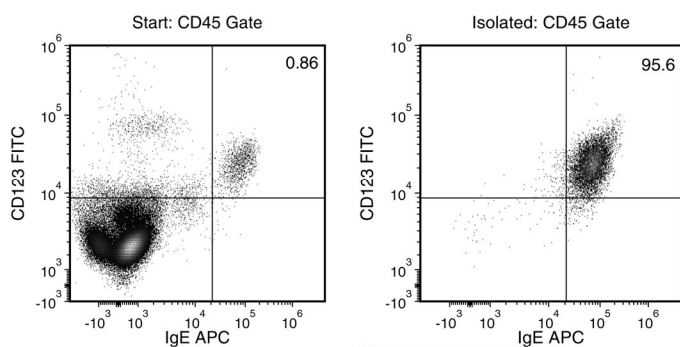
ASSESSING PURITY

For purity assessment of human basophils (CD123+IgE+ or CD123+CD203c^{low}) by flow cytometry use the following fluorochrome-conjugated antibody clones:

- Anti-Human CD123 (IL-3Ra) Antibody, Clone 6H6 (Catalog #60110), and
- Anti-human IgE antibody, clone MHE-18, or
- Anti-human CD203c antibody

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

Data



Starting with HetaSep™-processed human peripheral blood, the basophil content (CD45+CD123+IgE+) of the enriched fraction is typically 94.0 ± 2.5% (mean ± SD using the purple EasySep™ magnet). In the above example, the purities of the start and final enriched fractions are 0.86% and 95.6%, respectively.

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