EasySep™ Direct Human CTC Enrichment Kit

Negative Selection

Catalog #19657

For processing 100 mL whole blood

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 #DX20332 | Version 1_1_3

Description

Enrich circulating tumor cells (CTCs) directly from human whole blood by immunomagnetic negative selection.

The benefits of this kit include:

- > 99.9% red blood cell (RBC) depletion without the need for density gradient centrifugation, sedimentation, or lysis
- Up to 3 log depletion of normal hematopoietic cells
- Fast, easy-to-use and column-free
- Isolated cells are untouched

This kit targets hematopoietic cells and platelets for removal with antibodies recognizing CD2, CD14, CD16, CD19, CD45, CD61, CD66b, and Glycophorin A 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, cell culture, or DNA/RNA extraction.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Direct Human CTC Enrichment Cocktail	19657C	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.
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

For optimal RBC depletion, collect blood using heparin or acid citrate dextrose (ACD) as an anticoagulant. The use of K2EDTA or K3EDTA as an anticoagulant is not recommended.

For best recovery, use unprocessed human whole blood. Recovery of the desired enriched 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 enrichment 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

EasySep™ Buffer (Catalog #20144), 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™ Direct Human CTC Enrichment Kit Protocol

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	 **EasySep™ (Catalog #18000)**	**"The Big Easy" (Catalog #18001)** 
1	Collect sample within the volume range.	0.5 - 2 mL	1 - 7.5 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 Enrichment Cocktail to sample.	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	50 µL/mL of sample
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 double the volume for samples ≤ 1.25 mL • Top up to 2.5 mL for samples > 1.25 mL	• Top up to double the volume for samples ≤ 5 mL • Top up to 10 mL for samples > 5 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 10 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	Use a new 14 mL tube
7	Add RapidSpheres™ to the new tube containing the enriched cells and mix.	Use same volume as in step 4	Use same volume as in step 4
8	Place the tube (without lid) into the magnet and incubate.	RT for 10 minutes	RT for 10 minutes
9	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™ Direct Human CTC Enrichment Kit Protocol

		EASYSEP™ MAGNETS		
STEP	INSTRUCTIONS	EasyEights™ (Catalog #18103)		Easy 50 (Catalog #18002) 
		5 mL tube 	14 mL tube 	
1	Collect sample within the volume range.	0.5 - 2 mL	1 - 7.5 mL	5 - 25 mL
	Add sample to required tube (or plate when using the EasyPlate™ EasySep™ Magnet).	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)	50 mL (30 x 115 mm) conical tube (e.g. Catalog #38010)
2	Add Enrichment Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
3	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds	30 seconds
4	Add RapidSpheres™ to sample and mix.	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample
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 double the volume for samples ≤ 1.25 mL • Top up to 2.5 mL for samples > 1.25 mL	• Top up to double the volume for samples ≤ 5 mL • Top up to 10 mL for samples > 5 mL	Top up to double the volume
	Place the tube (without lid) into the magnet and incubate.	RT for 10 minutes	RT for 10 minutes	RT for 10 minutes
6	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 5 mL tube	Use a new 14 mL tube	Use a new 50 mL tube
7	Add RapidSpheres™ to the new tube containing the enriched cells and mix.	Use same volume as in step 4	Use same volume as in step 4	Use same volume as in step 4
8	Place the tube (without lid) into the magnet and incubate.	RT for 10 minutes	RT for 10 minutes	RT for 10 minutes
9	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	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 2 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 THE 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 other 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

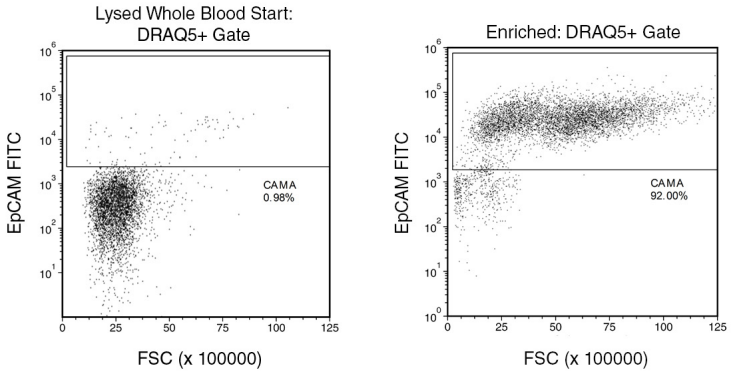
For purity assessment of CTCs by flow cytometry, use the following:

- Anti-Human CD326 (EpCAM) Antibody, Clone 5E11.3.1, FITC (Catalog #60147FI);
- DRAQ5™ Far-Red Fluorescence Live-Cell Permeant DNA Dye, eBioscience

NOTE: Assess purity on the DRAQ5™ positive cells to exclude debris.

Data

Starting with human whole blood from healthy donors, spiked with approximately 1% of CAMA cells (epithelial tumor cell line), the typical CTC (EpCAM+) content of non-lysed final enriched fraction is 79 ± 16 % (using the silver “Big Easy” EasySep™ Magnet; gated on DRAQ5™ for nucleated cells). Typically the log depletion of targeted CD45+ cells is 2.8 to 3.2.



In the above example, CAMA cells were seeded into whole blood at a starting frequency of 0.98%. The CAMA cell (EpCAM+) content of the enriched fraction is 92.02% with a 3.8 log depletion of CD45+ cells.