EasySep™ Human EGFR Positive Selection Kit

For processing 1 x 10^9 cells

Catalog #100-1131

Positive Selection

Document #10000019290 | Version 00

STEMCELL™ TECHNOLOGIES

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Description

Isolate highly purified EGFR+ cells from fresh peripheral blood mononuclear cells, washed leukapheresis, or cell culture samples by immunomagnetic positive selection.

- Fast and easy-to-use
- Up to 98% purity
- No columns required

This kit targets EGFR+ cells for positive selection with antibodies recognizing the EGFR surface marker. Desired cells are labeled with antibodies and magnetic particles and separated without columns using an EasySep™ magnet. Unwanted cells are simply poured off, while desired cells remain in the 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 EGFR Positive Selection Cocktail	300-0721	1 x 0.5 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A combination of monoclonal antibodies in PBS with 2% HPCD and 0.09% rHA. Includes an Fc receptor-blocking antibody.
EasySep™ Dextran RapidSpheres™ 50100	50100	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.

HPCD - 2-hydroxypropyl-β-cyclodextrin; PBS - phosphate-buffered saline; rHA - recombinant human albumin

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

Prepare a peripheral blood mononuclear cell (PBMC) suspension from whole blood by centrifugation over a density gradient medium (e.g. Lymphoprep™, Catalog #07801). For more rapid PBMC preparation, use the SepMate™ RUO (Catalog #86450/86415) or SepMate™ IVD* (Catalog #85450/85415) cell isolation tube.

If using previously frozen PBMCs, incubate the cells with DNase I Solution (Catalog #07900) at a concentration of 100 µg/mL at room temperature (15 - 25°C) for at least 15 minutes prior to labeling and separation. Filter aggregated suspensions through a 37 µm cell strainer (e.g. Catalog #27215) for optimal results.

After preparation, resuspend cells at 1 x 10^8 cells/mL in recommended medium.

* SepMate™ IVD is only available in select regions where it is registered as an In Vitro Diagnostic (IVD) device for the isolation of mononuclear cells (MNCs) from whole blood or bone marrow by density gradient centrifugation. In all other regions, SepMate™ is available for research use only (RUO).

WASHED LEUKAPHERESIS

Wash the peripheral blood leukapheresis sample by adding an equivalent volume of recommended medium or PBS containing 2% fetal bovine serum (FBS). Centrifuge at 300 x g for 10 minutes at room temperature. If red blood cell (RBC) lysis is desired, lyse with Ammonium Chloride Solution (Catalog #07800). If platelet removal is desired, centrifuge at 120 x g for 10 minutes with the brake off. Remove the supernatant and resuspend the cells at 1 x 10^8 cells/mL in recommended medium.

Recommended Medium

EasySep™ Buffer (Catalog #20144) or PBS containing 2% 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™ EasySep™ Human EGFR Positive Selection Kit Protocol

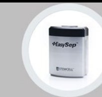
		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	 **EasySep™ (Catalog #18000)**	**"The Big Easy" (Catalog #18001)** 
1	Prepare sample at the indicated cell concentration within the volume range.	1 x 10^8 cells/mL 0.1 - 2 mL	1 x 10^8 cells/mL 0.25 - 8 mL
	Add sample to required tube.	5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007)	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	Add Selection Cocktail to sample. NOTE: Do not vortex cocktail.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 minutes
3	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
4	Add RapidSpheres™ to sample.	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 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 3 minutes	RT for 3 minutes
6	Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring off the supernatant. Remove the tube from the magnet; this tube contains the isolated cells.	Discard supernatant	Discard supernatant
7	Repeat steps as indicated.	Steps 5 and 6, two more times (total of 3 x 3-minute separations) • For low start samples (< 5%), increase separations by one for better purity (total of 4 x 3-minute separations) • For high start samples (≥ 50%), reduce separations by one for better recovery (total of 2 x 3-minute separations)	Steps 5 and 6, two more times (total of 3 x 3-minute separations) • For low start samples (< 5%), increase separations by one for better purity (total of 4 x 3-minute separations) • For high start samples (≥ 50%), reduce separations by one for better recovery (total of 2 x 3-minute separations)
8	Resuspend cells in desired medium. Be sure to collect cells from the sides of the 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 EGFR Positive Selection Kit Protocol

		EASYSEP™ MAGNETS		
		EasyEights™ (Catalog #18103)		
STEP	INSTRUCTIONS	5 mL tube	14 mL tube	Easy 50 (Catalog #18002)
1	Prepare sample at the indicated cell concentration within the volume range.	1 x 10^8 cells/mL 0.25 - 2 mL	1 x 10^8 cells/mL 0.5 - 8 mL	1 x 10^8 cells/mL 5 - 40 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)	50 mL (30 x 115 mm) conical tube (e.g. Catalog #38010)
2	Add Selection Cocktail to sample. NOTE: Do not vortex cocktail.	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 minutes	RT for 3 minutes
3	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds	30 seconds
4	Add RapidSpheres™ to sample.	100 µL/mL of sample	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 minutes	RT for 3 minutes
5	Add recommended medium to top up 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	Top up to: • 10 mL for samples ≤ 5 mL • 20 mL for samples > 5 - 10 mL • 30 mL for samples > 10 - 15 mL • 40 mL for samples > 15 - 20 mL • 50 mL for samples > 20 - 40 mL
	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) off the supernatant. Remove the tube, containing the isolated cells, from the magnet.	Discard supernatant	Discard supernatant	Discard supernatant
7	Add recommended medium to top up 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	Top up to: • 10 mL for samples ≤ 5 mL • 20 mL for samples > 5 - 10 mL • 30 mL for samples > 10 - 15 mL • 40 mL for samples > 15 - 20 mL • 50 mL for samples > 20 - 40 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
8	Carefully pipette** (do not pour) off the supernatant. Remove the tube, containing the isolated cells, from the magnet.	Discard supernatant	Discard supernatant	Discard supernatant
Continue on the next page.		Continue on the next page.	Continue on the next page.	Continue on the next page.

		EASYSEP™ MAGNETS		
STEP	INSTRUCTIONS (CONTINUED)	 EasyEights™ (Catalog #18103) 5 mL tube	14 mL tube 	Easy 50 (Catalog #18002) 
9	**Repeat steps as indicated.**	Steps 7 and 8 (total of 1 x 10-minute and 2 x 5-minute separations) • For low start samples (< 5%), increase separations by one for better purity (total of 1 x 10-minute and 3 x 5-minute separations) • For high start samples (≥ 50%), reduce separations by one for better recovery (total of 1 x 10-minute and 1 x 5-minute separations)	Steps 7 and 8 (total of 1 x 10-minute and 2 x 5-minute separations) • For low start samples (< 5%), increase separations by one for better purity (total of 1 x 10-minute and 3 x 5-minute separations) • For high start samples (≥ 50%), reduce separations by one for better recovery (total of 1 x 10-minute and 1 x 5-minute separations)	Steps 7 and 8 (total of 1 x 10-minute and 2 x 5-minute separations) • For low start samples (< 5%), increase separations by one for better purity (total of 1 x 10-minute and 3 x 5-minute separations) • For high start samples (≥ 50%), reduce separations by one for better recovery (total of 1 x 10-minute and 1 x 5-minute separations)
10	**Resuspend cells in desired medium. Be sure to collect cells from the sides of the 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 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]).

Notes and Tips

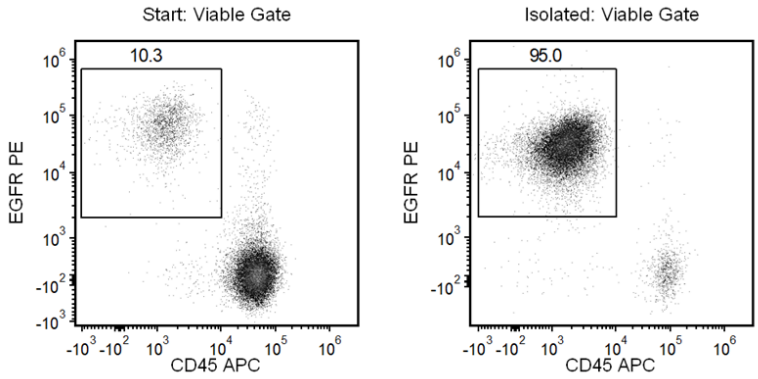
ASSESSING PURITY

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

- Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018), and
- Anti-human EGFR antibody, clone EGFR.1 (partially blocked)

NOTE: It is recommended to assess purity on viable cells by including a viability dye (e.g. Propidium Iodide [Catalog #75002]).

Data



Starting with 10% A549 cells spiked into human PBMCs, the EGFR+ cell content of the isolated fraction is typically 95.0 ± 2.8% (mean ± SD using the purple EasySep™ Magnet). In the above example, the purities of the start and final isolated fractions are 10.3% and 95.0%, respectively.