EasySep™ Human PSC-Derived Cardiomyocyte Enrichment Kit

Negative Selection

Catalog #17965

For processing 2.5 x 10^8 cells

STEMCELL™ TECHNOLOGIES

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Description

Isolate untouched and highly purified human pluripotent stem cell (PSC)-derived cardiomyocytes from cell cultures by immunomagnetic negative selection.

- Fast and easy-to-use
- Up to 99% purity
- Isolated cells are untouched

This kit targets non-cardiomyocytes 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 PSC-Derived Cardiomyocyte Enrichment Cocktail	17965C	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 0.1% rHA.
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

HUMAN PSC-DERIVED CARDIOMYOCYTES

Dissociate human PSC-derived cardiomyocytes using STEMdiff™ Cardiomyocyte Dissociation Kit (Catalog #05025). Refer to the associated Product Information Sheet (Document #21497) for detailed information on the recommended protocol. For more information, visit www.stemcell.com or contact us at techsupport@stemcell.com.

Filter aggregated suspensions through a pre-wetted 70 µm nylon mesh strainer (e.g. Catalog #27216) for optimal results. After preparation, resuspend cells at 2.5 x 10^7 cells/mL in recommended medium.

Recommended Medium

STEMdiff™ Cardiomyocyte Support Medium (Catalog #05027).

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™ Human PSC-Derived Cardiomyocyte Enrichment 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.	2.5 x 10^7 cells/mL 0.1 - 2 mL If starting with fewer than 2.5 x 10^6 cells, resuspend cells in 0.1 mL.	2.5 x 10^7 cells/mL 0.1 - 8 mL If starting with fewer than 2.5 x 10^6 cells, resuspend cells in 0.1 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.	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 < 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.

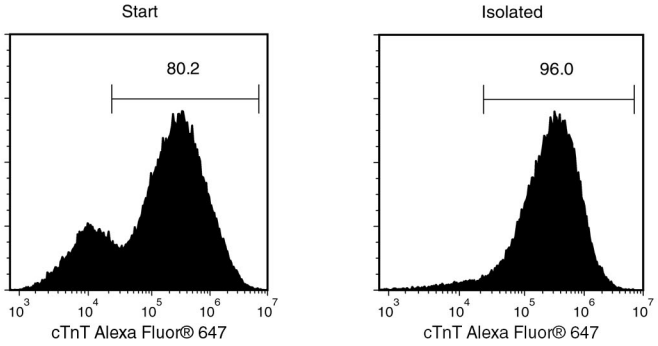
Notes and Tips

ASSESSING PURITY

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

- Anti-human cardiac troponin T antibody

Data



Starting with 1C cells differentiated using STEMdiff™ Cardiomyocyte Differentiation Kit (Catalog #05010), the cTnT+ cell content of the enriched fraction is typically 92.5 ± 7.6% (mean ± SD using the purple EasySep™ Magnet). In the above example, the purities of the start and final enriched fractions are 80.2% and 96.0%, respectively.