EasySep™ Release Human PSC-Derived Neural Crest Cell Positive Selection Kit

For processing 1 x 10^9 cells

Catalog #100-0047

Positive Selection

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Description

Isolate highly purified CD271+ cells from human pluripotent stem cell (PSC)-derived neural crest cell culture by immunomagnetic positive selection.

- Highly purified human PSC-derived neural crest cells isolated in less than 20 minutes
- No-wash removal of EasySep™ Releasable RapidSpheres™
- Compatible with downstream differentiation protocols

This kit targets human PSC-derived neural crest cells with antibody complexes recognizing the CD271 surface marker and EasySep™ Releasable RapidSpheres™. Desired cells are labeled with antibodies and magnetic particles, and separated without columns using an EasySep™ magnet. Unwanted cells are poured off, while desired cells remain in the tube. Then, bound magnetic particles are removed from the EasySep™-isolated human PSC-derived neural crest cells, which are immediately available for downstream applications such as flow cytometry or cell culture.

Following cell isolation with this EasySep™ Release kit, antibody complexes remain bound to the cell surface and may interact with Brilliant Violet™ antibody conjugates, polyethylene glycol-modified proteins, or other chemically related ligands.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Release Human PSC-Derived Neural Crest Cell Positive Selection Cocktail	300-0045	2 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 with 0.1% rHA.
EasySep™ Isolation Cocktail Enhancer	17900	1 x 1 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A solution that enhances the performance of the isolation cocktail.
EasySep™ Releasable RapidSpheres™ 50201	50201	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.
EasySep™ Release Buffer (Concentrate)	20165	3 x 1 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A buffer for release of Releasable RapidSpheres™ from cells following positive selection.

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

Trypsin-EDTA (0.25%; Catalog #07901) is recommended for preparation of a single-cell suspension from human PSC-derived neural crest cells, as follows:

1. Discard culture medium. Rinse each well of a 6-well plate with 2 mL DMEM/F-12.
2. Add 1 mL Trypsin-EDTA (0.25%) to each well.
3. Incubate at 37°C for 5 - 7 minutes, until the cells are easily dislodged from the bottom of the plate with gentle tapping.
4. Add 1 mL Soybean Trypsin Inhibitor, ACF (Catalog #07457) to each well.
5. Pipette up and down 3 - 4 times with a 1 mL pipette tip to dissociate to single cells.
6. Combine the single-cell suspension from each well into a 15 mL conical tube (e.g. Catalog #38009).
7. Centrifuge at 300 x g for 10 minutes, with the brake on low.
8. Remove supernatant and resuspend cells at 2.5 x 10^7 cells/mL in recommended medium.

NOTE: ACCUTASE™ (Catalog #07920) may be used in place of Trypsin. Refer to the Product Information Sheet for ACCUTASE™ for a cell detachment protocol.

Recommended Medium

RoboSep™ Buffer 2 (Catalog #20164) or PBS containing 0.5% bovine serum albumin (BSA) and 2 mM EDTA. Medium should be free of Ca++ and Mg++.

Directions for Use – Manual EasySep™ Protocol

See page 1 for Sample Preparation and Recommended Medium. Refer to Table 1 for detailed instructions regarding the EasySep™ procedure.

Table 1. EasySep™ Release Human PSC-Derived Neural Crest Cell Positive Selection Kit

STEP	INSTRUCTIONS	 EasySep™ (Catalog #18000)
1	Dilute Release Buffer (Concentrate) to prepare release buffer (1X).	Dilute 1 in 40 with recommended medium. NOTE: Release buffer (1X) must be prepared on the day of use. Refer to step 14 for required volume.
2	Prepare sample at the indicated cell concentration within the volume range.	2.5 x 10^7 cells/mL 0.1 - 2 mL
	Add sample to required tube.	5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007)
3	Add Selection Cocktail to sample.	50 µL/mL of sample
	Mix and incubate.	RT for 3 minutes
4	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds
5	Add Releasable RapidSpheres™ to sample.	50 µL/mL of sample
	Mix and incubate.	RT for 3 minutes
6	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
	Place the tube (without lid) into the magnet and incubate.	RT for 1 minute
7	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
8	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
9	Add Cocktail Enhancer to sample.	10 µL
	Mix and incubate.	RT for 3 minutes
10	Place the tube (without lid) into the magnet and incubate.	RT for 1 minute
11	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
12	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
	Place the tube (without lid) into the magnet and incubate.	RT for 1 minute
13	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
14	Add release buffer (1X) 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
	Mix and incubate.	RT for 3 minutes
15	Place the tube (without lid) into the magnet and incubate.	RT for 1 minute
16	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 (in the new tube) 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

EASYSEP™ RELEASE BUFFER

EasySep™ Release Buffer (Concentrate) is supplied as a 40X concentrate; release buffer (1X) must be prepared on the day of use. To prepare release buffer (1X), dilute an appropriate volume 1 in 40 with recommended medium. Refer to step 14 of Table 1 for required volume.

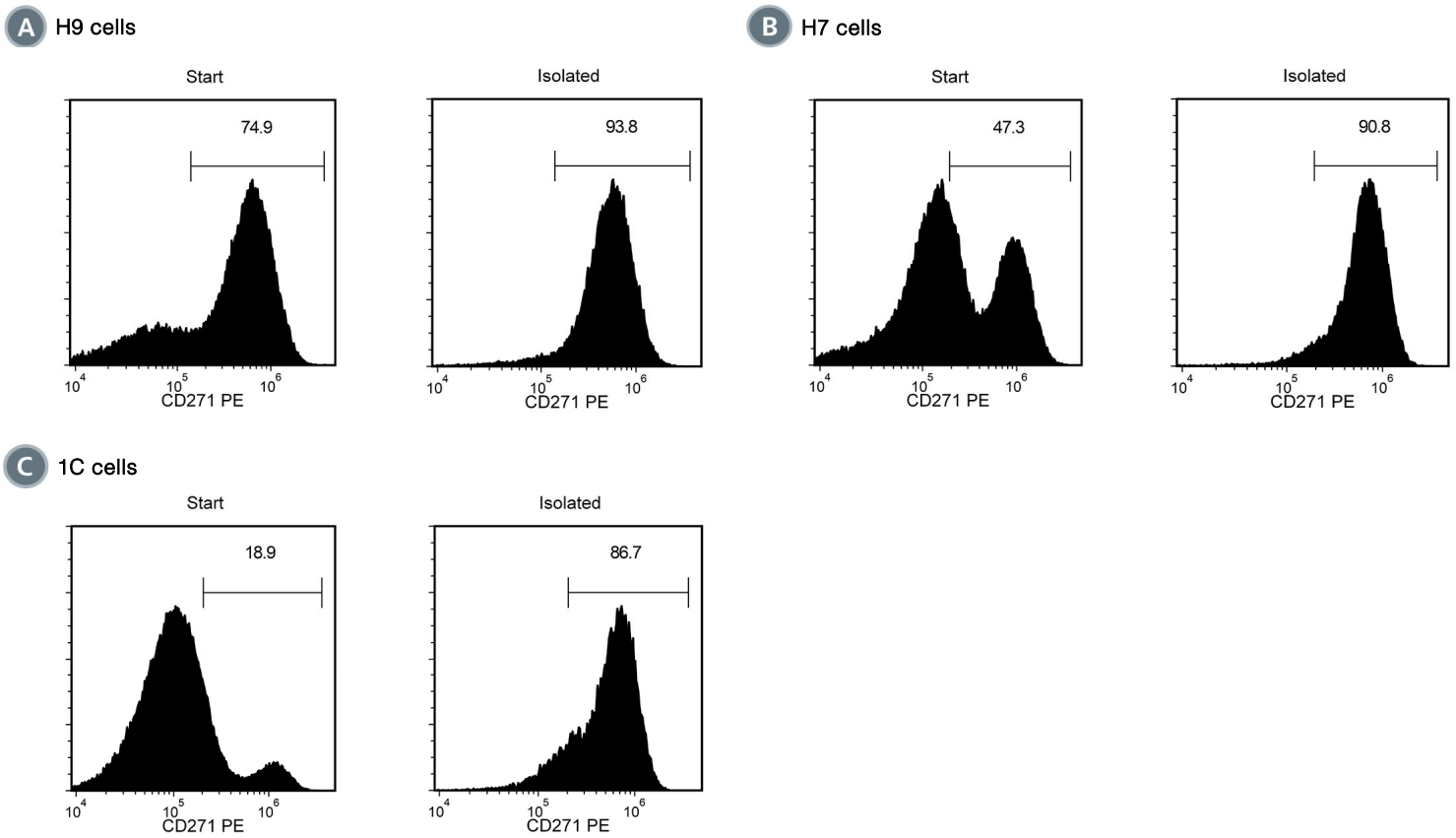
ASSESSING PURITY

For purity assessment of human PSC-derived neural crest cells by flow cytometry, use the following fluorochrome-conjugated antibody clone:
• Anti-human CD271 antibody, clone ME20.4

NOTE: Viability is measured by exclusion of DAPI (Hydrochloride; Catalog #75004), Propidium Iodide (Catalog #75002), or 7-AAD (7-Aminoactinomycin D; Catalog #75001).

NOTE: Brilliant Violet™ antibody conjugates should be carefully titrated on EasySep™ Release-isolated cells prior to analysis by flow cytometry or fluorescence microscopy. For purity assessment with Brilliant Violet™ antibody conjugates, use of BD Horizon Brilliant™ Stain Buffer is recommended to reduce non-specific interactions. For more information, refer to the manufacturer's instructions or contact us at techsupport@stemcell.com.

Data



Starting with a single-cell suspension of 1C, H7, or H9 cells differentiated using STEMdiff™ Neural Crest Differentiation Kit (Catalog #08610), the CD271+ cell content of the isolated fractions is typically 92.9 ± 6.3% (mean ± SD using the purple EasySep™ Magnet).
(A) Starting with H9 cells, the purities of the start and final isolated fractions are 74.9% and 93.8%, respectively.
(B) Starting with H7 cells, the purities of the start and final isolated fractions are 47.3% and 90.8%, respectively.
(C) Starting with 1C cells, the purities of the start and final isolated fractions are 18.9% and 86.7%, respectively.