EasySep™ hESC/hiPSC SSEA-4 Positive Selection Kit

Positive Selection

Catalog #18165

For processing 1 x 10^9 cells

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Scientists Helping Scientists™ | WWW.STEMCELL.COM
TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713
INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM
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Description

Isolate highly purified human embryonic stem (ES) and induced pluripotent stem (iPS) cells by immunomagnetic positive selection.

- Fast and easy-to-use
- Up to 99% purity
- No columns required

This kit targets SSEA-4+ cells for positive selection with an antibody recognizing the SSEA-4 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™ hESC/hiPSC SSEA-4 Positive Selection Cocktail	18165C.1	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™ Magnetic Nanoparticles Positive Selection	18150	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.

PBS - phosphate-buffered saline

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

Sample Preparation

ES or iPS CELL CULTURES

To prepare a single-cell suspension from ES or iPS cell cultures:

1. Rinse the cells once with D-PBS (Without Ca++ and Mg++; Catalog #37350).
2. Add ACCUTASE™ (Catalog #07920) to the cells (e.g. 1 - 2 mL ACCUTASE™ per well of a 6-well plate).
3. Incubate at 37°C for 5 - 10 minutes.
4. Use a pipette to break up any remaining clumps of cells, and transfer the cell suspension to a new tube.
5. Rinse the well with PBS and add this solution to the same new tube.
6. Centrifuge.
7. Discard the supernatant and resuspend cells at 1 x 10^8 cells/mL in recommended medium.

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 Table 1 for detailed instructions regarding the EasySep™ procedure for each magnet.

Table 1. EasySep™ hESC/hiPSC SSEA-4 Positive Selection Kit Protocol

		EASYSEP™ MAGNET
STEP	INSTRUCTIONS	 **EasySep™ (Catalog #18000)**
1	**Prepare sample at the indicated cell concentration within the volume range.**	1 x 10^8 cells/mL 0.1 - 0.5 mL NOTE: If starting with fewer than 1 x 10^7 cells, resuspend cells in 0.1 mL
	Add sample to required tube.	5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352058)
2	**Add Selection Cocktail to sample.**	100 µL/mL of sample
	Mix and incubate.	2 - 8°C for 15 minutes
3	**Mix Magnetic Particles.** **NOTE: Particles should appear evenly dispersed.**	Pipette up and down more than 5 times
4	**Add Magnetic Particles to sample.**	50 µL/mL of sample
	Mix and incubate.	2 - 8°C for 10 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
	Place the tube (without lid) into the magnet and incubate.	RT for 10 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
7	**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 5 minutes
8	**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
9	**Repeat steps as indicated.**	Steps 7 and 8 (total of 1 x 10-minute and 2 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
OPTIONAL ADDITIONAL SEPARATION for PURITY **NOTE: This will improve purity but may reduce recovery.**		---
11	**Repeat steps as indicated.**	Steps 7 and 8 (one additional 5-minute separation)
12	**Resuspend cells in desired medium. Be sure to collect cells from the sides of the tube.**	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 SSEA+ cells by flow cytometry use the following fluorochrome-conjugated antibody clone:

- Anti-Human SSEA-4 Antibody, Clone MC-813-70 (Catalog #60062)

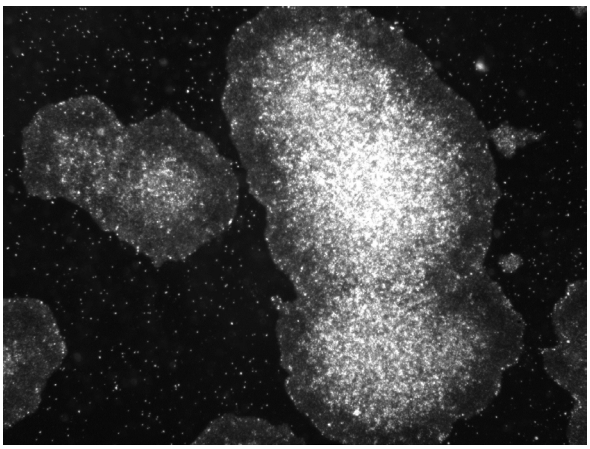
NOTE: Flow cytometry analysis of the isolated cells may show slightly increased side scatter relative to the start sample.

SSEA4+ CELL DEPLETION

EasySep™ hESC/hiPSC SSEA-4 Positive Selection Kit can also be used to deplete SSEA-4+ cells. Contact us at techsupport@stemcell.com to request a copy of the protocol.

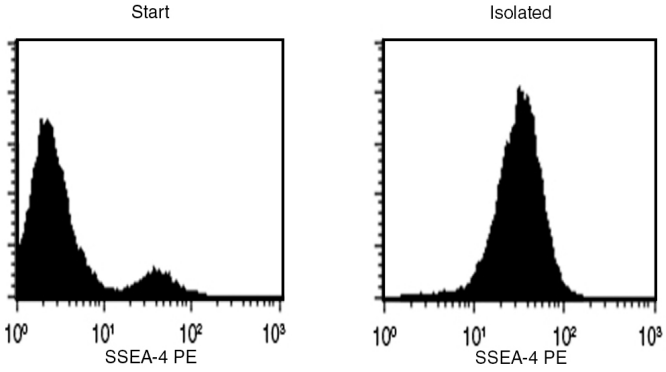
Data

CULTURE OF CELLS ISOLATED BY SSEA-4 POSITIVE SELECTION



SSEA-4+ selected cells were cultured in mTeSR™1 (Catalog #05850) on Corning® Matrigel® for 3 passages, using Dispase (1 U/mL; Catalog #07923) for passaging. The Dispase passaging procedure is outlined in the Technical Manual: Maintenance of Human Pluripotent Stem Cells in mTeSR™1 (Document #29106).

TYPICAL EASYSEP™ SSEA-4 POSITIVE SELECTION PROFILE



Starting with a single-cell suspension containing approximately 10% human ES cells or iPS cells, the SSEA-4+ content of the isolated fraction is typically 85 - 99%. In the above example, the purities of the start and final isolated fractions are 11.8% and 98.8%, respectively.

References

Andrews PW et al. (1982) Cell-surface antigens of a clonal human embryonal carcinoma cell line: morphological and antigenic differentiation in culture. Int J Cancer 29(5): 523–31.

Andrews PW et al. (1996) Comparative analysis of cell surface antigens expressed by cell lines derived from human germ cell tumours. Int J Cancer 66(6): 806–16.

Draper JS et al. (2002) Surface antigens of human embryonic stem cells: changes upon differentiation in culture. J Anat 200(Pt 3): 249–58.