Selection of SSEA4+ Human Pluripotent Stem Cells From a Mixed Population

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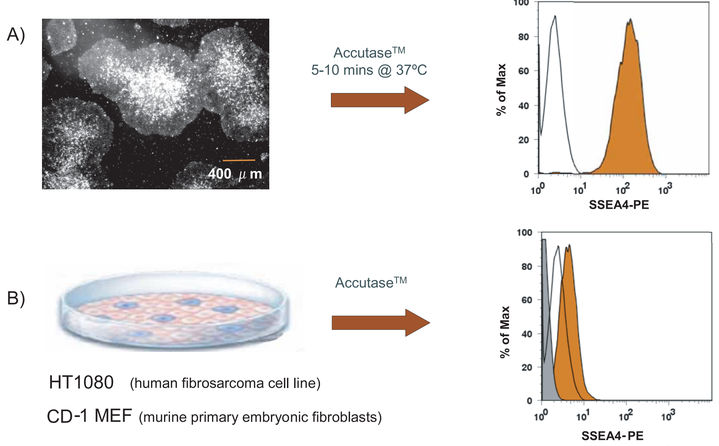
Abstract

Undifferentiated human pluripotent stem cells (PSCs) express SSEA-4 on their cell surface, and this expression is downregulated with differentiation, making it a reliable marker of pluripotency. We describe a rapid and simple method for the enrichment of PSCs by positive selection, that yields reliably high cell purities and recoveries. PSCs were isolated using immuno-magnetic, column-free positive selection (EasySep®), based on expression of SSEA-4. Importantly, the selected cells were able to reattach to Matrigel® coated plates in mTeSR®1 medium, and maintained characteristic undifferentiated morphology for at least 3 passages in mTeSR®1 following selection.

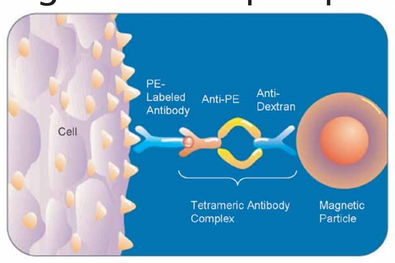
Methods

Figure 1: Mixed Suspension of PSCs and control cells for use in EasySep®



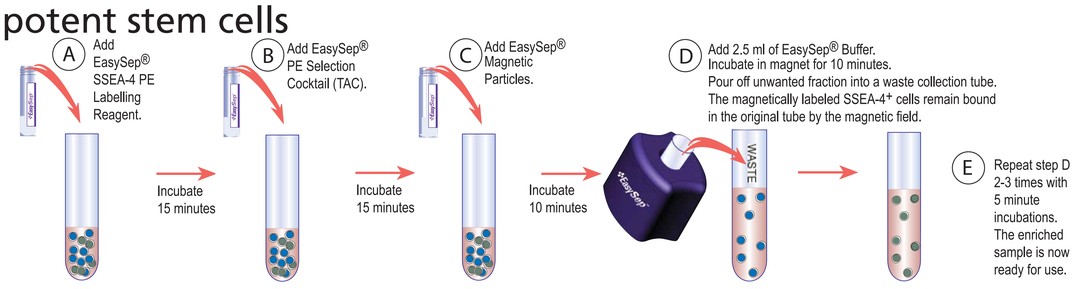
A) Single cell suspensions of H9 hES cells were obtained by incubating with Accutase®, and were confirmed by FACS analysis to be SSEA-4 positive (right: orange=H9 stained with SSEA-4 PE; white=isotype control). B) Single cell suspensions of non-PSC control cells: HT1080 fibrosarcoma cells or CD-1 murine embryonic fibroblasts (MEF) were obtained by incubating with Accutase® and were confirmed to be SSEA-4 negative (right: grey=MEF; orange=HT1080; white=isotype control).

Figure 2: EasySep® labeling of human pluripotent stem cells



SSEA-4 expressing cells are specifically labeled with anti-SSEA-4 PE antibody, followed by bispecific tetrameric antibody complex (TAC) and dextran-coated magnetic particles. Using the EasySep® magnet, the desired SSEA-4+ cells can then be retained, and unwanted cells removed from the suspension.

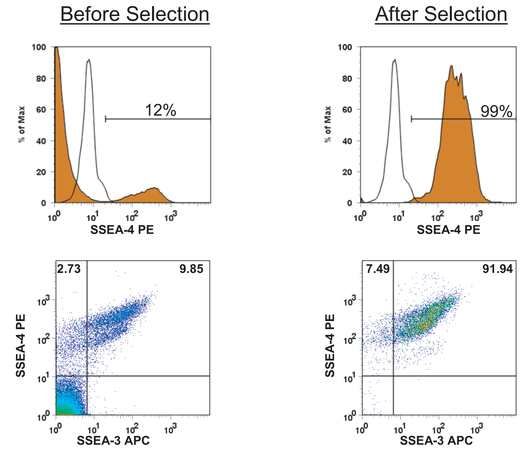
Figure 3: EasySep® procedure for column-free enrichment of human pluripotent stem cells



The EasySep® SSEA-4 PSC positive selection kit is designed to isolate human PSCs which express SSEA-4 (STEMCELL Cat #18165). (A-C) Single cell suspension from PSC-containing culture is incubated sequentially with EasySep® SSEA-4 PE Labelling Reagent, EasySep® PE Selection Cocktail (TAC), and EasySep® Magnetic Particles. D) After incubation in the magnet for 10 minutes, unlabelled cells are poured off into a waste collection tube while the magnetically labeled SSEA-4+ cells are held in the original tube by the EasySep® magnet. E) 2-3 additional rounds of magnetic cell separation enable the isolation of highly purified PSCs that are ready to use in functional assays. The entire procedure requires only 60 minutes.

Results

Figure 4: SSEA-4 EasySep® Enrichment FACS Profiles



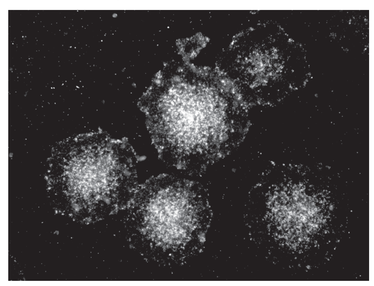
Cells were mixed at 1:9 ratio (10^6 PSCs and $9x10^6$ non-PSCs), to simulate a mixed PSC population. Shown here is a representative experiment starting with a mixed population containing 12% SSEA-4+ cells and resulting in 99% SSEA-4+ cells after 4 rounds of separation (top: orange= SSEA-4 PE; white= isotype control, 1.24%+). Selected cells also co-stain with pluripotency marker SSEA-3 (bottom).

Table 1: Purity and recovery of SSEA-4+ PSCs enriched by EasySep® positive selection using three or four rounds of selection

	# of Replicates	Purity (%)	Recovery (%)
3 rounds of selection	**6**	**72 ± 6**	**63 ± 26**
4 rounds of selection	**7**	**88 ± 4**	**28 ± 15**

Values expressed as mean ± SD
Purity and recovery determined by flow cytometry. All samples gated on viable (PI negative) cells.

Figure 5: Selected PSCs maintain undifferentiated colony morphology for at least 3 passages after EasySep® selection



PSCs obtained after EasySep® selection from a mixed population of 10% hES cells and 90% MEF or HT1080 cells were plated onto Matrigel®-coated plates in mTeSR®1 medium. The cells were able to establish colonies with typical hES morphology, lacking overt signs of differentiation or noticeable fibroblast outgrowths.

Conclusions

- PSCs can be rapidly isolated from mixed populations using column-free positive selection.
- High recovery and high purity can be achieved, by performing 3 or 4 rounds of EasySep® separation.
- Enriched PSCs can be used to re-seed PSC cultures to give normal colonies.

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