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Application Note

Generation of Neural Stem Cells from AlphaSTEM™ Cultured Pluripotent Stem Cells

Introduction

Patient-derived induced pluripotent stem cells (iPSCs) offer exciting potential for disease modeling, regenerative medicine, and therapeutic development. In order to fully realize the potential of iPSCs, it is critical to develop culture and differentiation methodologies that result in the most biologically relevant terminal cells (e.g. Neurons).

Minerva Biotechnologies is the first company to generate human naïve state induced pluripotent stem cells (iPSCs) using a single, naturally occurring human stem cell growth factor, $NME7_{AB}$. Naïve stem cells have several advantages over current stem cells, called 'primed' state cells. Scientists believe that because these earlier stem cells have a "clean slate", they are more easily directed to develop into functional mature cells. Using Minerva's AlphaSTEM™ Culture System, differentiation efficiency is increased and the resulting cells have enhanced functionality.

Materials and Methods

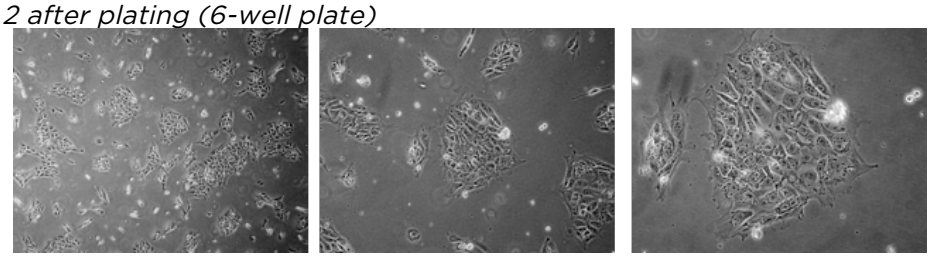
In this study, iPSCs were expanded in the AlphaSTEM™ Culture System which generates superior naïve stem cells which can be enzymatically passaged as single cells, expand 10X in 4 days, and are karyotypically stable. The cells then underwent neural induction using the PSC Neural Induction Medium from Thermo Fisher Scientific. The PSC Neural Induction Medium is a serum-free medium that provides high efficiency neural induction of human pluripotent stems cells (PSCs) in only 7 days.

Culturing iPSCs using AlphaSTEM™ Naive hPSC Medium

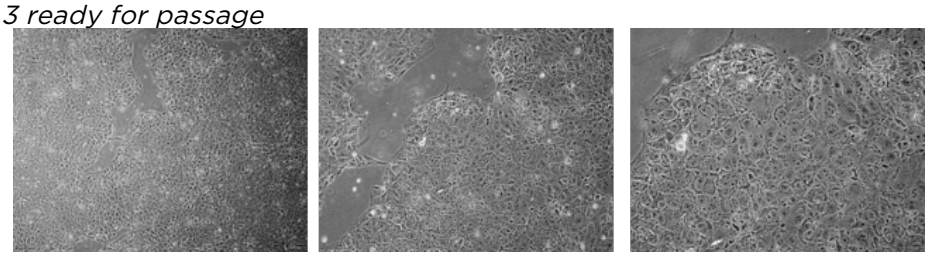
AlphaSTEM™ Naïve hPSC Medium is a serum-free and feeder-free medium formulated for the generation and expansion of naïve pluripotent stem cells.

Figure 1: Representative images of AlphaSTEM™ grown cells seeded at 100,000 cells per well

Day 2 after plating (6-well plate)



Day 3 ready for passage



1. Day 0: Thaw iPSCs by slowly adding 9 mL pre-warmed AlphaSTEM™ medium containing 10 µM Y-27632 Rho kinase I inhibitor at 37°C.
2. Plate cells by adding 1 mL cells (100,00cells) to 2 mL pre-warmed AlphaSTEM™ medium containing 10 µM Y-27632 Rho kinase I inhibitor.
3. Rock plates to distribute cells evenly and leave undisturbed for 48 hours.
4. Culture in 37°C, 5% CO2, 5% O2.

5. Day 2: After first 48 hours, gently change medium every 24 hours.
6. Cells will grow as a monolayer. Passage cells at 80% confluence (~3-4 days).
7. To harvest, dissociate using 1 mL TrypLE (~3 min. at 37°C) and neutralize with AlphaSTEM™ medium. Centrifuge at 250 x g, 5 minutes. Resuspend in 1 mL AlphaSTEM™ medium.
8. On fresh AlphaSTEM™ Culture Matrix coated plates, seed cells in a single-cell suspension at 10,000 cells/cm2 (100,000 per well of a 6-well plate) in AlphaSTEM™ medium. Repeat steps 3 through 8, expanding cells with each passage. Expect 8-10-fold expansion in 4 days.

Neural Induction Protocol

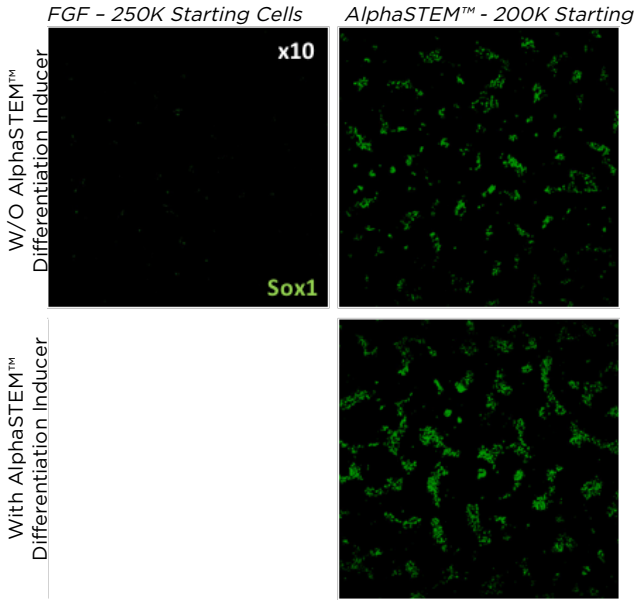
1. Pre-warm complete PSC Neural Induction Medium to room temperature.
2. On day 0 of neural induction (about 24 hours after PSC splitting), PSCs should be at 15–25% confluency. Add PSC Neural Induction Medium and AlphaSTEM™ Differentiation Inducer to the cells. Return the plates into 37°C incubator (humidified, 5% CO_2)
3. On day 2 of neural induction, the morphology of cell colonies should be uniform. Mark all non-neural differentiated colonies, if any, and remove such unwanted colonies with a Pasteur glass pipette or pipette tip.
4. On day 4 of neural induction, cells will be reaching confluency. Any non-neural differentiated colonies should be marked and removed.
5. On day 6 of neural induction, cells should be at near maximal confluence.
6. On day 7 of neural induction, NSCs (P0) are ready to be harvested.

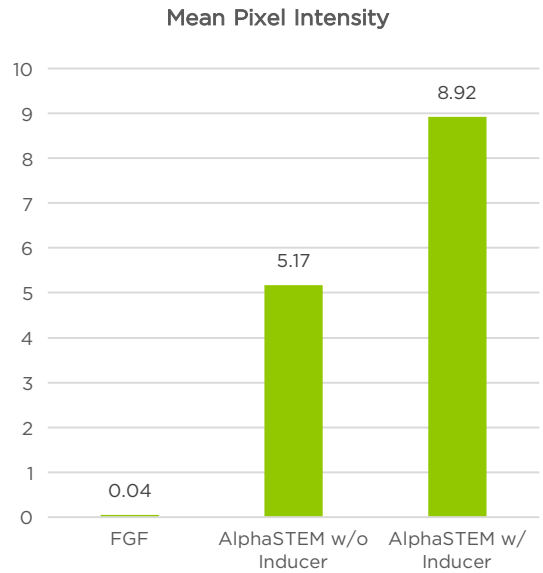
Results

Naïve stem cells do not have DNA methylation marks that commit the cells to certain developmental decisions. The result is differentiation of cells grown in AlphaSTEM™ Naive hPSC Medium is superior to that of FGF grown cells in terms of quality, quantity and purity.

Increased Differentiation Efficiency

AlphaSTEM™ Naive hPSC Medium increases directed differentiation to neural cells by 150X over FGF media. When AlphaSTEM™ Differentiation Inducer is used, the increase is 250X.





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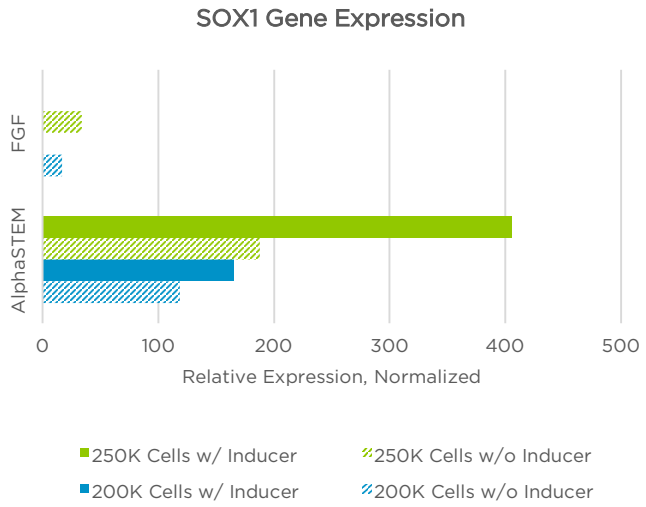
Improved Marker Expression



AlphaSTEM™ Differentiation Inducer increases expression of SOX1 in AlphaSTEM™ Naive hPSC Medium grown stem cells.

Increased Cell Yield

AlphaSTEM™ Naive hPSC Medium increases final NSC yield compared to FGF based media. Data shows fold expansion during induction protocol was independent of starting cell number.

FGF Primed Cells	AlphaSTEM™ Naïve Cells	
	W/O AlphaSTEM™ Differentiation Inducer	With AlphaSTEM™ Differentiation Inducer
7.8X	25X	31X

Conclusions

In this study, we demonstrated that using this system results in more efficient neural induction with fold expansion increasing to 25X compared to 7.8x (>300% increase) and a population of cells with 12-fold higher expression of SOX1 at Day 7 compared to the same number of starting FGF source cells. The addition of AlphaSTEM™ Differentiation Inducer further increases the yield giving a 4-fold increase in total cell number and an increase in the number of SOX1 expressing cells by 2-fold.

The Minerva AlphaSTEM™ Culture System was developed to provide a superior alternative to FGF-based cell culture. Using AlphaSTEM™ Naive hPSC Medium, iPSC generation is simpler, cell expansion is faster, differentiation efficiency is increased and the resulting cells have enhanced functionality.

References

1. A Primitive Growth Factor, NME7$_{AB}$, Is Sufficient to Induce Stable Naïve State Human Pluripotency; Reprogramming in This Novel Growth Factor Confers Superior Differentiation.
Carter MG, Smagghe BJ, Stewart AK, Rapley JA, Lynch E, Bernier KJ, Keating KW, Hatziioannou VM, Hartman EJ, Bamdad CC, Stem Cells. 2016 Apr;34(4):847-59.

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