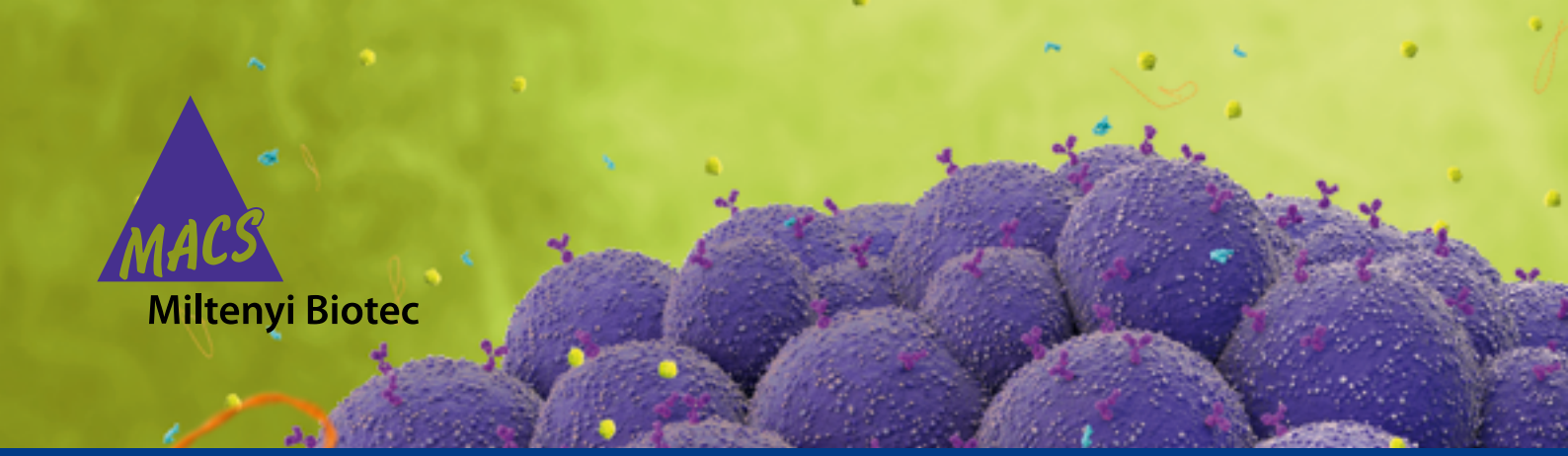

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Neural differentiation of human pluripotent stem cells

Performance of StemMACS™ iPS-Brew XF in the maintenance and neural differentiation of human pluripotent stem cells

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Abstract

A high-quality medium with robust performance is imperative for the culture of human pluripotent stem cells (hPSCs). Here, we have tested the StemMACS™ iPS-Brew XF and evaluated its performance in the maintenance and differentiation of hPSCs in feeder-free conditions. Our evaluation includes maintenance of the pluripotent state as well as differentiation into the neural lineage.

Background

StemMACS iPS-Brew XF is a xeno-free cell culture media formulation for the maintenance and expansion of hPSCs under feeder-free conditions. The formulation supports rapid adaptation of feeder-based cell cultures to a feeder-free environment and is compatible with commonly used cell attachment matrices, e.g., Matrigel® or vitronectin. StemMACS iPS-Brew XF enables robust and efficient expansion of human embryonic stem (ES) cells or induced pluripotent stem (iPS) cells over multiple passages while maintaining a pluripotent phenotype as well as pluripotent differentiation potential. StemMACS iPS-Brew XF also allows for rapid culture re-initiation of PSC cultures after cryopreservation.

Materials

StemMACS iPS-Brew XF (# 130-104-368)

- Basal Medium, (500 mL), lot numbers: 5140812077, 5150217276, 5150618252, 5150415897
- Supplement, lot numbers: 5140616435, 5140821097, 5150527138, 5150416360

Antibodies

Anti-TRA-1-60-Vio® 488 Live Cell Stain, human (# 130-106-872)

Anti-TRA-1-81-Vio 488 Live Cell Stain, human (# 130-106-873)

Neural induction medium

• KnockOut™ DMEM	415 mL
• KnockOut SR	75 mL
• Glutamax™ (100×)	5 mL
• Non-essential amino acids (100×)	5 mL
• Beta-mercaptoethanol (1000×)	0.5 mL
• SB431542 (10 mM)	500 µL (1000×)
• LDN-193189 (10 mM) (100,000×; if making small amount of medium, dilute LDN-193189 stock by 100× first, then use as 1000×)	5 µL

N2 medium

• DMEM/F12	245 mL
• Glucose (20%)	1.94 mL
• N-2 supplement (100%)	2.5 mL
• Insulin (10 mg/mL)	0.5 mL

After combining N2 medium and neural induction medium, add LDN-193189 to 100 nM.

Methods

Maintenance of pluripotency

Human iPSCs, either line MS-3 or line 530-1 (P8), were cultured on Matrigel in StemMACS™ iPS-Brew XF. iPSCs were passaged with EDTA approximately every 4 days with a 1:4 split ratio.

Neural differentiation protocol

hiPSCs maintained in StemMACS iPS-Brew XF were single-cell dissociated and differentiated into the neural lineage according to standard protocols[1]. Briefly, cells were dissociated with Accutase, resuspended, and plated with the addition of 10 µM ROCK Inhibitor (Y27632) for 24 hours at a seeding density of 75,000 cells/well of a 12-well plate. After the removal of ROCK inhibitor, cells were fed with StemMACS iPS-Brew XF medium for 3 days. On day 4 after seeding the cells, neural differentiation was induced in neural induction medium containing SB431542 (10 µM) and LDN-193189 (100 nM).
On day 6 of differentiation, SB431542 was removed and 25% N2 medium was added.
On day 7, cells were either fixed for analysis (see fig. 3) or continued to be cultured for further differentiation.
Lastly, we examined the differentiation potential of hPSCs grown in StemMACS iPS-Brew XF by differentiation into the neuroectodermal lineage followed by immunofluorescence imaging (figs. 3 and 4).
On day 8, the proportion of N2 medium was increased to 50%, cells were fed daily.
On day 10, the proportion of N2 medium was increased to 75%, cells were fed daily.
On day 12, cells were fixed for analysis of β-III tubulin by immunocytochemistry.

Results

We have evaluated maintenance of the pluripotent state by phase contrast imaging and live cell staining (figs. 1 and 2). The multiple lots that have been tested have performed consistently, however the data shown below were obtained with StemMACS iPS-Brew XF, lot #5150618252 for basal medium and 5150416360 for supplement.

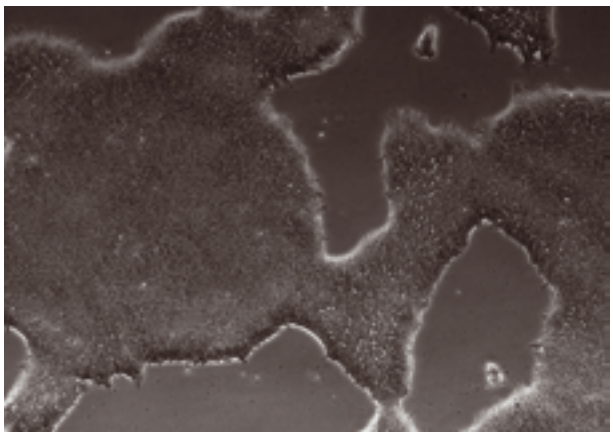

Figure 1: Phase contrast images of MS-3 cell line grown in StemMACS iPS-Brew XF.

The TRA-1-60 and TRA-1-81 Vio® 488 antibodies allow for real-time pluripotency analysis of cells grown in StemMACS iPS-Brew XF (fig. 2).

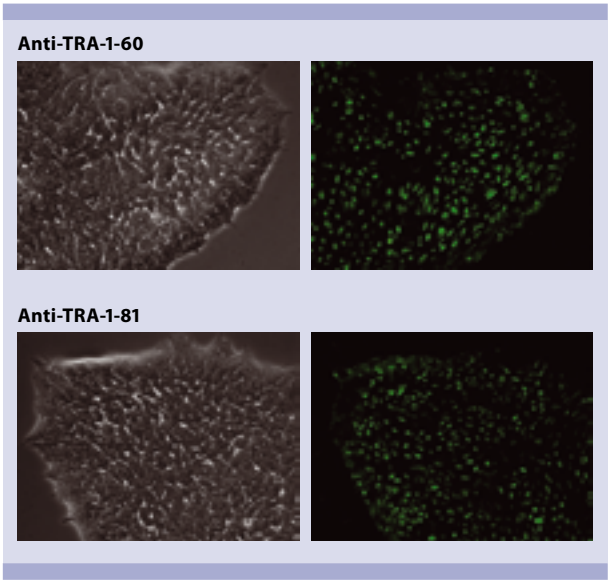

Figure 2: Live-cell pluripotency analysis of hPSCs.
Fluorescence images of iPSC line 530-1 with the Anti-TRA-1-60-Vio 488 and Anti-TRA-1-81-Vio 488 Live Cell Stain antibodies.

Lastly, we examined the differentiation potential of hPSCs grown in StemMACS™ iPS-Brew XF by differentiation into the neuroectodermal lineage followed by immunofluorescence imaging (figs. 3 and 4).

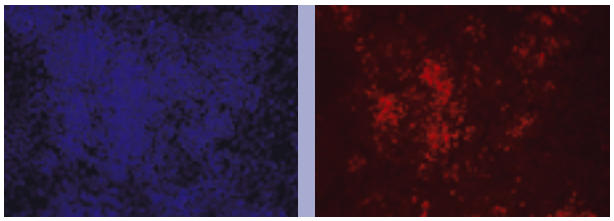

Figure 3: Analysis of the ectodermal marker PAX6. PAX6-positive, neuroectodermal cells at day 7 of differentiation were detected by immunofluorescence (red) in MS-3 cell line. Nuclei in the same field of view were counter-stained with DAPI (blue); 10× magnification.

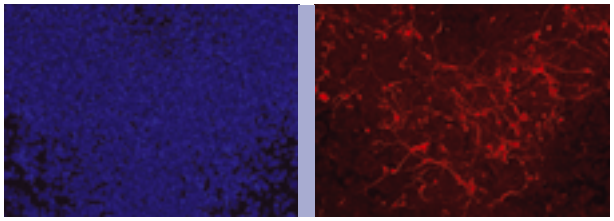

Figure 4: Neural differentiation of hPSCs. Differentiation of MS-3 iPSCs was analyzed by immunofluorescence on day 12, after staining with β-III tubulin antibody (red) and DAPI (blue).

Conclusion

StemMACS iPS-Brew XF is an efficient, cost-effective medium for the maintenance of hPSCs. We have tested the following lots and the results are consistent across all lots tested:

- Basal Medium lot numbers: 5140812077, 5150217276, 5150618252, 5150415897
- Supplement lot numbers: 5140616435, 5140821097, 5150527138, 5150416360.

StemMACS iPS-Brew XF performs as robustly as commercially available alternatives and the ease-of-use is superior to home-brew and traditional feeder coculture methods. We highly recommend StemMACS iPS-Brew XF to grow hPSCs.

Reference

1. Chambers, S.M. *et al.* (2009) Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. Nat. Biotechnol. 27: 275–280.

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