TECHNICAL BULLETIN

LONG-TERM CULTURE OF HUMAN EMBRYONIC AND INDUCED PLURIPOTENT STEM CELLS IN mTeSR™1

Introduction

Undifferentiated human pluripotent stem cells have the potential for unlimited expansion with the retention of normal karyotype and the ability to generate cells of all three germ layers — endoderm, mesoderm and ectoderm.

Human pluripotent stem cell research has been advanced significantly by recent developments in culture conditions which support the proliferation and culture of human embryonic and induced pluripotent stem cells (hESCs and hiPSCs) in completely defined, feeder-independent conditions over long culture periods. Traditional methods of deriving and maintaining hESCs used feeder cells and fetal bovine serum. However, the use of feeders with human pluripotent stem cells causes quality control difficulties due to undefined components (batch variability). This raises concerns over the transmission of foreign pathogens or expression of immunogenic antigens. Furthermore, use of feeder cells is labor-intensive and limits large-scale culture of these cells.

STEMCELL Technologies has developed mTeSR™1, a complete, serum-free and defined medium for the feeder-independent maintenance of hESCs and hiPSCs in culture. mTeSR™1 has been used in the derivation of over 50 independent hESC and hiPSC lines in 26 countries, and is the most widely published feeder-independent maintenance medium for human pluripotent cells.

This technical bulletin describes the use of mTeSR™1 for the long-term culture of hESCs and hiPSCs. The reliability and convenience of mTeSR™1 enables consistent, reproducible data to be generated quickly, thereby augmenting the clinical potential of pluripotent cells, studies of human development systems and the screening of small molecules or drugs.

hESCs and hiPSCs maintained in mTeSR™1:

- are phenotypically homogeneous and karyotypically normal
- express high levels of multiple antigens associated with pluripotency: Oct-3/4, SSEA-3, SSEA-4, TRA-1-60, TRA-1-81
- maintain expression of POU5F1 (Oct-3/4), NANOG and ZFP42 (REX-1) mRNAs
- form teratomas containing derivatives of endo-, meso-, and ectodermal lineages
- can be differentiated into functional mature cell types in vitro
- require no adaptation period (i.e. no period of mixed media dilutions) when transferred from feeder-based culture

Advantages of using mTeSR™1:

Consistency

- Elimination of undefined components
- No variability from feeder cells

Reproducibility

- Standardization of hESC and hiPSC culture methods
- Referenced in over 80 peer-reviewed scientific publications

Time and Labor Savings

- Elimination of feeder cell preparation and medium conditioning

Convenience

- Complete medium formulation (no additional supplements necessary)

TABLE 1. mTeSR™1 has been tested and published extensively for the long-term maintenance and expansion of various cell lines, including those listed below (*Anecdotal customer evidence).

hESC AND hiPSC LINES MAINTAINED IN mTeSR™1			
H1, H9, H7	H13*, H14, H15*, H16*	BG01, BG02, BG03, BG04*	Shef1*, Shef4*
HESM01*, HESM02*, HESM03*, HESM04*	HS237, HS239, HS360, HS401	HUES1, HUES3, HUES6, HUES8*, HUES9	Regea 06/015*
MA01*, CA1*, CA1T*, KCL1*, Man1*, MEL-1*, MEL-2*	NCL-3, HSF-6*, HES2, HES3 (ES03), HES4*	Banked at WISC bank at WiCell: iPS-DF19-9-11T.H, iPS-DF19-9-7T, iPS-DF4-3-7T, iPS-DF6-9-9T.B, iPS(Foreskin)-1 (Clone 1), iPS IMR90-1, iPS(IMR90)-4 (Clone 4)	iPSC(IMR90)-3, MSC-iPSC1

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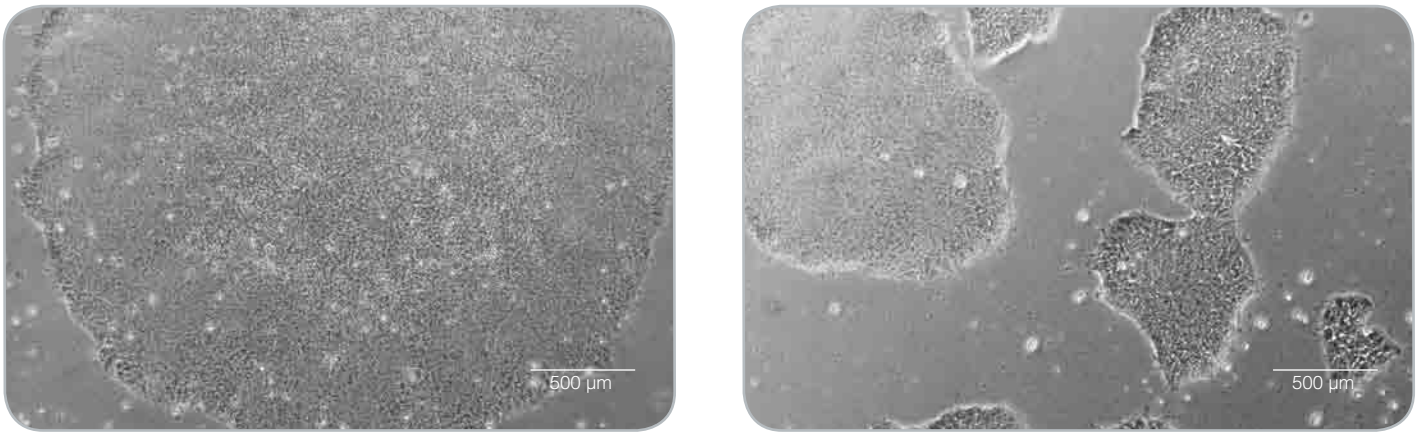
Maintenance Data

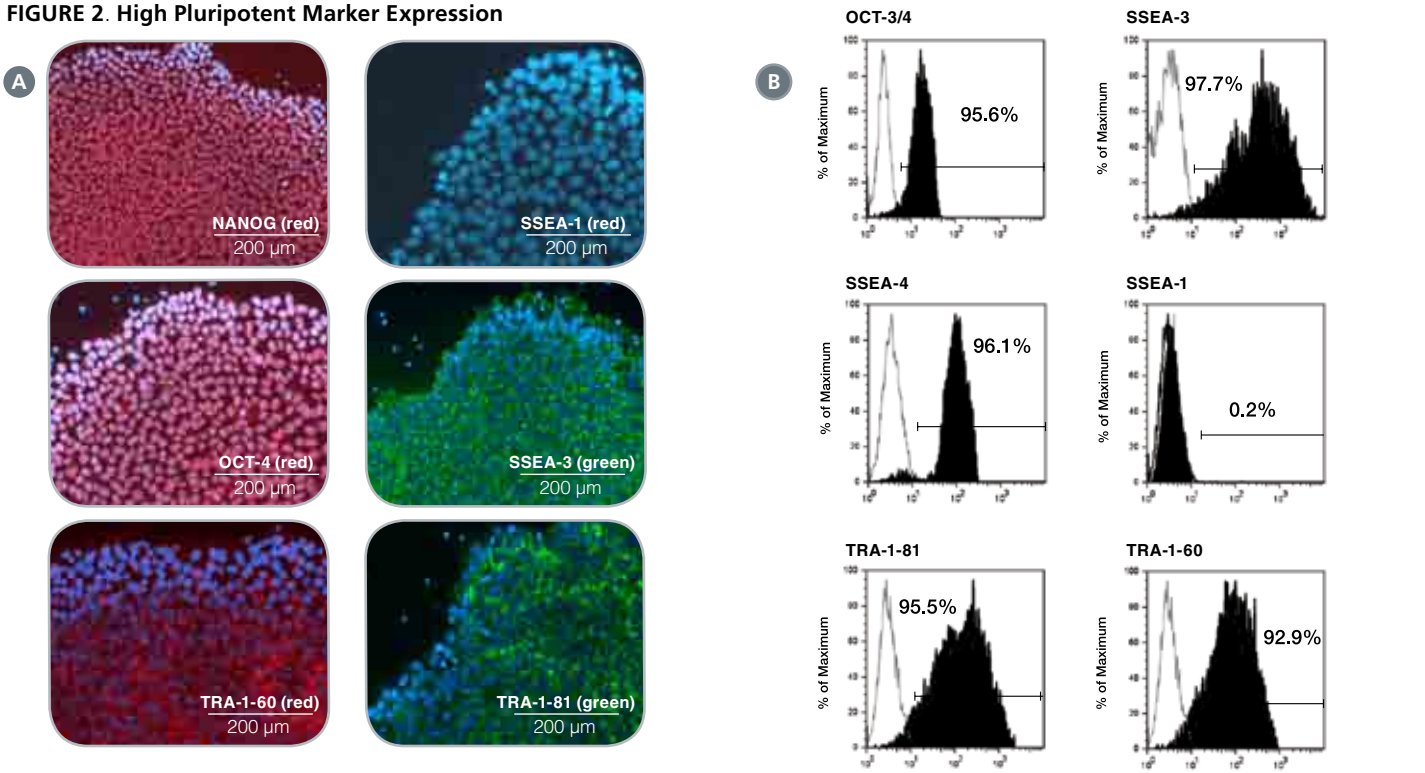
The following data was generated using standard mTeSR™1 protocols, which are detailed in the complimentary Technical Manual "Maintenance of hESCs and hiPSCs in mTeSR™1", available from www.stemcell.com. Where indicated, data was kindly provided by Heidi Hongisto from the Regea Institute for Regenerative Medicine, University of Tampere.

FIGURE 1. Normal hESC and hiPSC Morphology



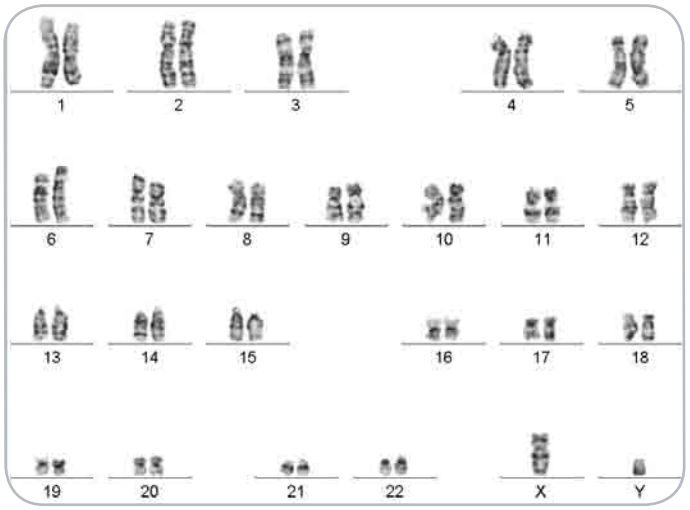
Regea 06/015 cells grown in mTeSR™1 for 25 and 22 passages respectively, show that the hESCs retain normal morphology. Images courtesy of Heidi Hongisto from the Regea Institute for Regenerative Medicine, University of Tampere.

FIGURE 2. High Pluripotent Marker Expression



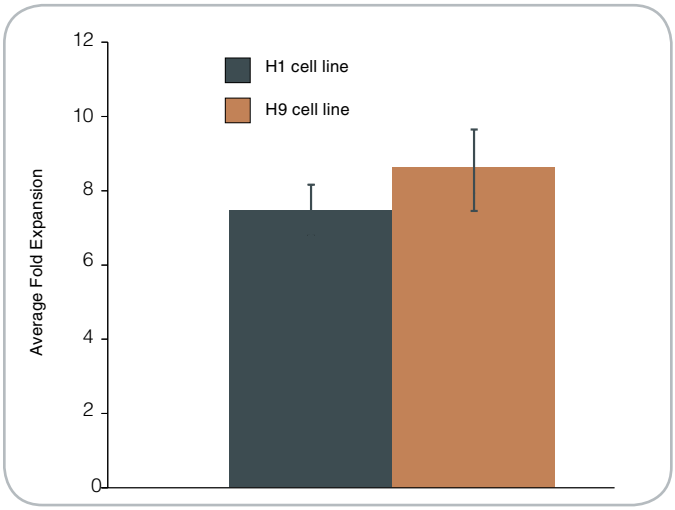
Immunofluorescent staining (A) of characteristic pluripotent markers in Regea 06/015 hESCs grown in mTeSR™1 for > 30 passages demonstrates high marker expression (DAPI - blue). Flow cytometric analysis (B) on H9 hESCs grown in mTeSR™1 for 17 passages confirms high pluripotent marker expression levels and low expression of the differentiation marker SSEA-1. Fluorescent images are courtesy of Heidi Hongisto from the Regea Institute for Regenerative Medicine, University of Tampere.

FIGURE 3. Normal Chromosomal Stability



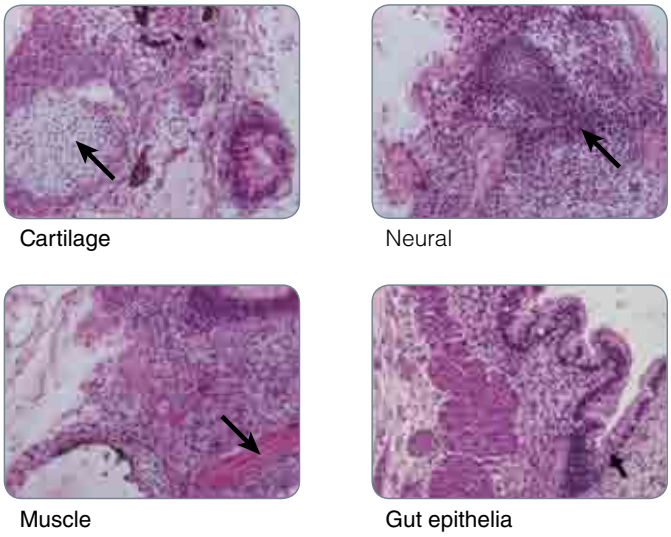
G-band karyotype analysis of Regea 06/015 hESCs maintained in mTeSR™1 at passage 35 confirm long-term chromosomal stability.* Data courtesy of Heidi Hongisto from the Regea Institute for Regenerative Medicine, University of Tampere.

FIGURE 4. Consistent Expansion Capacity



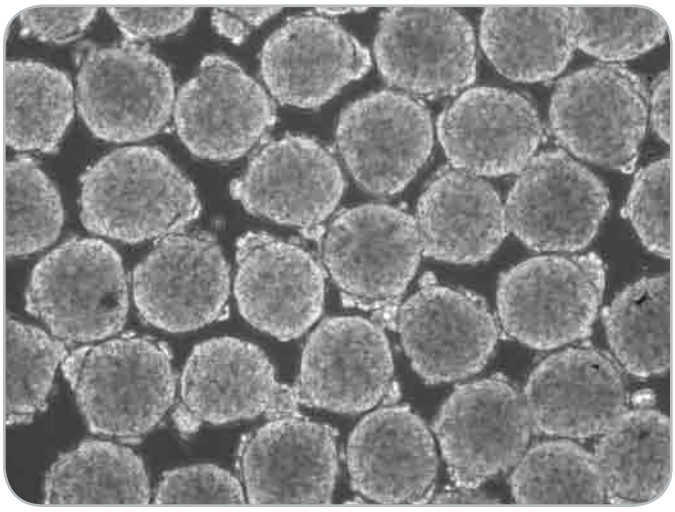
H1 and H9 hESCs were expanded in mTeSR™1 for 19 and 18 passages respectively. Cultures show 7- to 10-fold expansion consistently across passages.

FIGURE 5. hESCs and hiPSCs Cultured in mTeSR™1 are Pluripotent



H9 hESCs were cultured for 6 passages in mTeSR™1 then injected subcutaneously into immunocompromised mice. The resulting teratoma contained cell types from all 3 germ layers. Representative tissue types are shown.

FIGURE 6. hESCs and hiPSCs Cultured Long-Term in mTeSR™1 Readily Form Embryoid Bodies (EBs)



H9 hESCs maintained in mTeSR™1 form uniformly-shaped and size-controlled EBs when generated with an AggreWell™400 plate.

*Note: Normal karyotype has been shown in H1 hESCs maintained in mTeSR™1 after as many as 76 passages (Data not shown).

Tips for successful long-term hESC and hiPSC culture in mTeSR™1:

A) Preparation of hESC and hiPSC clumps for passaging

Preparation of a uniform suspension of suitably-sized clumps for passaging is very important for the successful culture of hESCs and hiPSCs. If the clumps are too large, an increased rate of differentiation may occur within the colonies. If the clumps are too small, with many single cells present, cell survival (and thus colony survival) will be compromised. Following the enzymatic passaging (with dispase) and washing steps, one or two gentle draws with a micropipetter and 1 mL micropipette tip or a 2 mL serological pipette should be sufficient to generate appropriately-sized clumps for passaging (approximately 50 - 60 µm in diameter). If the clumps are the correct size, most will hang in suspension after this step. If there are large clumps that rapidly sink to the bottom of the tube, perform one or two more gentle draws with the micropipetter or pipette.

B) Density

Culture density is critical to maintaining hESCs and hiPSCs in mTeSR™1. Cultures that are either too sparsely or too densely populated can lead to differentiation. At the time of passage, the plate should be approximately 75% confluent (i.e. most colonies should barely touch each other in the dish). Adjust plating density and/or split ratios to achieve a balance between having too much space between colonies and having a confluent culture. Generally, an acceptable plating density would be approximately 150 colonies per well of a 6-well plate.

C) Differentiation in maintenance culture

It is important that the starting culture be of high quality and be primarily undifferentiated. Cultures that have been compromised or have large amounts of differentiated cells will maintain those characteristics after transition into mTeSR™1. If necessary, areas of differentiation can be selectively removed by scraping off or aspirating before passaging. Morphologic hallmarks of differentiation should appear in less than 20% of colonies in a healthy culture.

D) Feeding regimen

hESCs and hiPSCs generally require a daily medium change for optimal growth. Some hESC and hiPSC lines will tolerate occasional double feeding (adding twice the required volume of medium on alternate days), but this practice is not recommended more than once per week.

PRODUCT	QUANTITY	CATALOG #
mTeSR™1	500 mL	05850
	10 x 500 mL	05870
	25 x 500 mL	05875
	1L	05857

Support Products

PRODUCT	CATALOG #
AggreWell™400 plates	27845 / 27945
AggreWell™800 plates	27865 / 27965
AggreWell™400Ex plates (COMING SOON!)	27840/ 27940
AggreWell™ Medium	05893
Oct 3/4 Antibody, Clone 40	01550 / 01551
SSEA-1 Antibody, Clone MC-480	01552
SSEA-3 Antibody, Clone MC-631	01553
SSEA-4 Antibody, Clone 813-70	01554
STEMcircles™-LGNSO	05820
TRA-1-60 Antibody, Clone TRA-1-60	01555
TRA-1-81 Antibody, Clone TRA-1-81	01556
TRA-2-49 Antibody, Clone TRA-2-49/6E	01557
TRA-2-54 Antibody, Clone TRA-2-54/2J	01558
mFreSR™ Defined Cryopreservation Medium	05855 / 05854
Dispase (1 mg/mL)	07923

Select References on Applications for Cells Cultured in mTeSR™1

Feeder-independent derivation of hiPSCs
Yu et al., Science 318:1917-1920, 2007
Sun et al., PNAS 106:15720-15725, 2009
Chan et al., Nature Biotech 27:1033-1037, 2009

Feeder-independent culture of hiPSCs
Eminli et al., Nat Genet 41:968-976, 2009
Yu et al., Science 318:1917-1920, 2008

Culture of hESCs in a system using rhVitronectin
Braam et al., Stem Cells 26:2257-2265, 2008
Prowse et al., Biomaterials 31:8281-8288, 2010

Upscaling hESC culture
Azarin SM and Palecek SP. Biochem Eng 48:378-384, 2010
Singh et al. Stem Cell Res 4:165-179, 2010

Directed hESC differentiation towards different therapeutic lineages
Hematopoiesis: Lu et al., Regen Med 3:693-704, 2008
Cardiomyocytes: Raya et al., Cold Spring Harb Symp Quant Biol 73:127-135, 2008
Neural: Porayette et al., J Biol Chem 284:23806-23817, 2009
Endothelial: Yu et al., PLoS One. 2009 Sep 15;4(9):e7040

Banking of hiPSC
WiCell International Stem Cell Bank, Madison, Wisconsin

For a list of select mTeSR™1 publications, please visit: www.stemcell.com/mTeSR1publications