

mTeSRTM1 and mFreSR[®]: Serum-Free, Defined and Feeder-Independent Solutions for hESC and iPSC Culture and Cryopreservation

Jennifer L Moody¹, Debbie King¹, Michael O'Connor², Min Lu^{1,2}, M. Kardel², Andrea Tegzes¹, Cindy Miller¹, Connie Eaves², Allen Eaves^{1,2}
¹STEMCELL Technologies Inc., Vancouver, BC V5Z 1B3, ²Terry Fox Laboratory, British Columbia Cancer Research Centre, Vancouver, BC V5Z 1L3

Introduction

The continued success of pluripotent stem cell research is ultimately dependent on access to reliable, standardized and defined reagents that allow consistent maintenance of undifferentiated cell cultures and preparation of high quality cryopreserved cell stocks. While feeder-dependent culture systems and/or serum-containing media have previously been the best options for human embryonic stem cell (hESC) culture and cryopreservation, there is a recognized need for culture conditions with less variability and more defined components to aid in elucidating the properties of hESC and how they are controlled.

mTeSRTM1 is a fully defined, feeder-independent medium based on work published by leaders in the field of hESC research.¹ mTeSRTM1 allows for robust and consistent, undifferentiated expansion of human pluripotent cells, including embryonic stem cell lines and induced pluripotent stem cell lines. mFreSR[®] is a serum-free and defined cryopreservation medium developed for use with mTeSRTM1 cultures. When used with standard laboratory equipment, mFreSR[®] allows for successful cryopreservation of hESC as measured by the undifferentiated, robust subsequent expansion of cells post thaw. Together these products provide researchers with complete media solutions for maintaining high quality pluripotent cultures.

Results

Figure 1. Classic morphology of undifferentiated hESC and induced pluripotent stem cells (iPSC) in mTeSRTM1 medium.

Cells grown in mTeSRTM1 (STEMCELL catalog # 05850) show the typical undifferentiated morphology consisting of a homogenous distribution of cells exhibiting high nucleus to cytoplasm ratio. (A) H1p43 (16 passages (≅ 96 days) in mTeSRTM1), (B) MSC-iPSC1 p21 (4 passages (≅ 37 days) in mTeSRTM1).

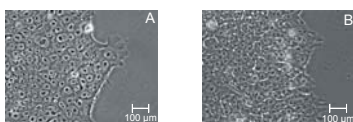


Figure 2. Long term robust expansion of cells cultured in mTeSRTM1.

The H9 and H1 cell lines were maintained in mTeSRTM1 on Matrigel[™] for approximately 5 months and were passaged on average, every 6 days. Cells were passaged according to the methods described in the mTeSRTM1 manual (http://www.stemcell.com/technical/29106_mTeSR1.pdf). The average fold expansion of H9 and H1 in mTeSRTM1 was calculated by dividing the number of clumps yielded at passaging by the number of clumps seeded for 18 and 19 passages, respectively, ±SEM.

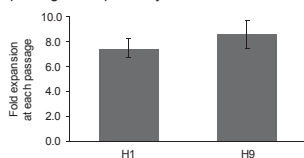


Figure 3. Karyotypic stability of long-term mTeSRTM1 cultures.

mTeSRTM1 is able to maintain karyotypically normal hESCs over long periods as demonstrated by G-banding of (A) H1p186 and (B) H9p64 hESCs grown in mTeSRTM1 for 76 and 44 passages, respectively. Data kindly provided by Tennifer Ludwig, University of Wisconsin-Madison.

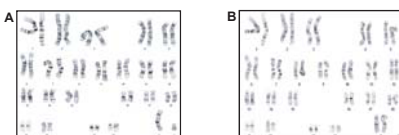


Figure 4. The colony forming cell (CFC) frequency of mTeSRTM1 cultured cells is enhanced by the addition of Y-27632 Rock inhibitor.

Single cell suspensions of H1 or H9 cells were generated with Accutase (STEMCELL catalog # 07920) and plated in mTeSRTM1 at approximately 20,000 cells per cm² without Y-27632 (baseline) or at approximately 2,000 cells per cm² with the addition of 10µM Y-27632 (Calbiochem). Data was calculated by dividing the number of alkaline phosphatase positive colonies (>30 cells) on day 7 by the number of cells seeded ± SEM. This data indicates a CFC increase of approximately 19-26 fold in the presence of Y-27632, similar to previously published reports^{2,3}.

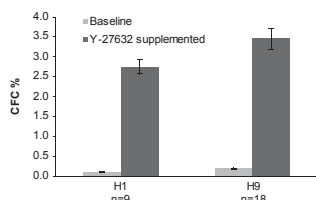


Figure 5. Pluripotency of mTeSRTM1 cultured cells demonstrated *in vivo* by teratoma formation.

Cells maintained in mTeSRTM1 for multiple passages retain the ability to form tissues representing all three germ layers. hESCs were harvested as aggregates and injected sub-cutaneously into NOD/SCID mice. The resulting grafts were harvested 8 to 10 weeks after injection and were fixed, embedded in paraffin, sectioned and stained. Hematoxylin and eosin stained sections of teratomas show (A) cartilage (mesoderm; arrow) (B) neural epithelium with neuropil (ectoderm; arrow). (C) goblet cells with crypt formation (endoderm; arrow).

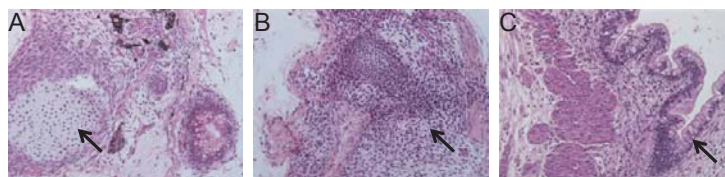
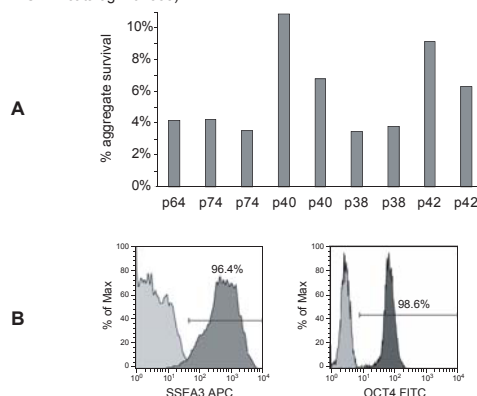


Figure 6. Cells cryopreserved in mFreSR[®] yield undifferentiated cultures upon thaw and expansion in mTeSRTM1.

H9 cells grown in mTeSRTM1 were frozen at various passages in mFreSR[®] (STEMCELL catalog # 05855) and were stored in liquid nitrogen vapor phase for a range of times, from 2 days to 7 months. Vials were thawed independently into mTeSRTM1 on Matrigel[™] with the number of clumps plated at thaw recorded. (A) Approximately 6 days post thaw the number of morphologically undifferentiated colonies was enumerated and is expressed as a percentage of clumps plated. (B) Representative FACS profiles of H9 22 days post thaw demonstrate high expression of the pluripotency markers Oct3/4 (STEMCELL catalog # 01550) and SSEA-3 (STEMCELL catalog # 01553).



Summary

Cells grown in mTeSRTM1:

- Retain the classic undifferentiated morphology associated with pluripotent cells
- Expand in a robust, reliable manner - on average 6-9 fold per passage
- Maintain karyotypic stability over prolonged passaging
- Are responsive to the increased survival conferred by the compound Y-27632²
- Are capable of multilineage differentiation
- Can be cryopreserved in mFreSR[®] and subsequently yield high quality, undifferentiated cultures

References

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WWW.STEMCELL.COM

IN NORTH AMERICA

TOLL-FREE TEL: 1 800 667 0322 TOLL-FREE FAX: 1 800 567 2899

TEL: 1 604 877 0713 FAX: 1 604 877 0704 EMAIL: INFO@STEMCELL.COM

IN EUROPE

TEL: +33 (0)4 76 04 75 30 FAX: +33 (0)4 76 18 99 63 EMAIL: INFO.EU@STEMCELL.COM

IN AUSTRALIA

TOLL-FREE TEL/FAX: 1 800 060 350 TEL: 07 5474 5042 EMAIL: INFO.AUS@STEMCELL.COM