

# HUMAN EMBRYONIC STEM CELL Culture Environments

Advancing the Field of **Human Embryonic Stem Cell** Research

## mTeSR™ 1

### Maintenance Medium for Human Embryonic Stem Cells

- + Feeder-Independent
- + WiCell™ Formulation<sup>1</sup>
- + Serum-Free
- + Defined

## BD Matrigel™ hESC-qualified Matrix

- + Qualified as  
mTeSR™ 1-Compatible
- + No Pre-Screening Required

+ Oct-3/4 immunostaining of a human ES cell colony in mTeSR™ 1



**StemCell Technologies Inc**  
The Cell Experts™



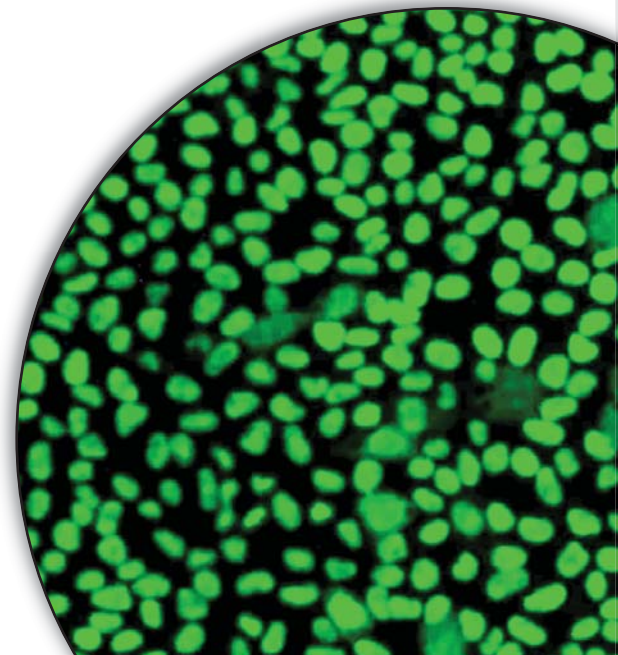
# HUMAN EMBRYONIC STEM CELL Culture Environments

## LEVERAGING COLLABORATIVE EXPERTISE to Advance Human Embryonic Stem Cell Research

BD Biosciences, StemCell Technologies, and the WiCell™ Research Institute have established a strategic collaboration to develop optimized, feeder-independent cell culture environments for human embryonic stem (hES) cell research, including serum-free defined media and qualified surfaces. Through the strength of this collaboration, BD Biosciences and StemCell Technologies will provide products that are superior in quality, performance and reliability in order to facilitate and standardize the field of hES cell research.

WiCell™ Research Institute is a world-leader in the area of hES cell research with a mission to expand the frontiers of science and medicine. Focused on enhancing and expanding the study of hES cells, WiCell™ Research Institute continuously supports efforts to unlock the potential of this leading-edge scientific field. BD Biosciences and StemCell Technologies now offer a number of products, under license from WiCell™ Research Institute, to enable researchers to explore this great potential.

Because of their ability to differentiate into multiple, clinically-useful cell types, tremendous hope is associated with the potential application of hES cells in cell therapy and regenerative medicine. Historically, hES cell derivation and culture techniques utilized serum and/or mouse embryonic fibroblast (MEF) feeder layers<sup>2</sup>. This has led to concerns about the future clinical use of these cells due to the potential contamination with animal pathogens, proteins and macromolecules. Furthermore, the use of serum and MEF feeders introduce significant sources of variability. The maintenance of MEF feeders for use with hES cells is also inconvenient, expensive and time-consuming. For these reasons, researchers have shown considerable interest in the development of feeder-independent, serum-free, defined conditions for culturing hES cells.



## **MEDIA + SURFACES = Complete Cell Environments**

StemCell Technologies has commercially developed and optimized WiCell™ Research Institute's mTeSR™1 medium formulation<sup>1</sup> to standardize feeder-independent hES cell culture. mTeSR™1 is complete, defined and serum-free, and has been designed to maintain and expand hES cells in an undifferentiated state when used with BD Matrigel™ Matrix as a substrate. It does not require any further addition of growth factors. Undifferentiated hES cells maintained in mTeSR™1 express high levels of pluripotency markers such as Oct-3/4 and SSEA-3. Pluripotency of cells maintained in mTeSR™1 has been demonstrated by the ability of the cells to differentiate into all three germ layers in the teratoma assay.

An ideal culture environment for hES cells consists of both a serum-free, defined medium and a cell culture surface specifically qualified for hES cells.

BD Biosciences has developed an optimized surface for your hES cell research. BD Matrigel™ Matrix, coupled with a variety of culture media, has been widely accepted as an alternative substrate to feeder-dependent culture of hES cells<sup>3,4</sup>. BD Matrigel™ Matrix is a reconstituted basement membrane isolated from the Engelbreth-Holm-Swarm (EHS) mouse sarcoma. This matrix is predominantly composed of laminin, collagen IV, entactin, and heparin sulfate proteoglycan.

BD Biosciences now offers BD Matrigel™ hESC-qualified Matrix for use with mTeSR™1. Each batch has been qualified as mTeSR™1-compatible by StemCell Technologies, thus eliminating the need for time-consuming screening, in order to provide the reproducibility and consistency essential for your hES cell research. The mTeSR™1 formulation and BD Matrigel™ Matrix have been shown to be a successful combination for culturing different WiCell™ hES cell lines for up to 20 passages<sup>5</sup>.

mTeSR™1 and BD Matrigel™ hESC-qualified Matrix, a high quality medium and surface combination, create the first complete environment to support feeder-independent expansion of hES cells.

*Advancing the Field of*  
**Human Embryonic  
Stem Cell Research** *with:*

## **mTeSR™1**

### **Maintenance Medium for Human Embryonic Stem Cells**

- + Feeder-independent
- + Published WiCell™ formulation
- + Serum-free
- + Defined medium components
- + Complete medium, no addition of growth factors required
- + Manufactured and performance tested by world-leader in specialized media standardization

## **BD Matrigel™ hESC-qualified Matrix**

- + Optimized surface for hES cell culture
- + Qualified as mTeSR™1-compatible by StemCell Technologies

### **Benefits of mTeSR™1 and BD Matrigel™:**

- + Trusted formulation from WiCell™, the field leader in human embryonic stem cell research
- + Eliminate variabilities associated with feeder-dependent cultures
- + Increase efficiency by avoiding the maintenance of feeder cells
- + Reduce time and costs associated with screening medium and surface components
- + Ensure high quality, reliability, and reproducibility with defined, qualified components
- + Culture hES cells under standardized conditions

## Characterization of Undifferentiated hES Cells

Undifferentiated hES cells grow as compact, multicellular colonies of cells with a high nucleus-to-cytoplasm ratio, prominent nucleoli and a distinct colony border (see *Figure 2*). Healthy hES cell colonies will have “phase-bright” centers when viewed under a phase contrast microscope. Loss of border integrity, the presence of flat, cobblestone-like cells, or holes in the middle of a colony, are all signs of differentiation.

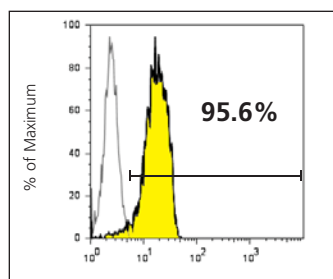
A complementary method to assess undifferentiated hES cell colonies is by using fluorescence activated cell sorting (see *Figure 1*) or immunohistochemical staining (see *Figure 3*). High expression of cell surface antigens such as the glycolipid antigens SSEA-3 and SSEA-4 and the keratan sulphate-related antigens Tra-1-60 and Tra-1-81 generally correlate with the undifferentiated state<sup>7</sup>. Expression of Octamer-3/4 (Oct-3/4), a homeodomain transcription factor of the POU family, also correlates with the pluripotent state. Therefore, Oct-3/4 is frequently used as a marker for undifferentiated cells.

*Figure 3* shows immunohistochemical staining of an undifferentiated hES cell colony using a mouse monoclonal Oct-3/4 antibody followed by an anti-mouse secondary antibody, conjugated to fluorescein isothiocyanate (FITC).

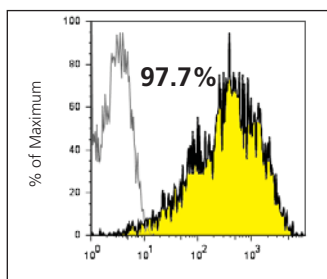
One of the defining features of embryonic stem cells is pluripotency defined by the potential to differentiate into each of the three germ layers: ectoderm, mesoderm, and endoderm. This differentiation potential can be examined *in vivo* by teratoma formation<sup>1,6</sup>. The teratoma assay involves injection of hES cells into an immunocompromised mouse. After four to twelve weeks, pluripotent cells will give rise to teratomas containing tissues representative of the three germ layers. The resultant tumors can be excised and analyzed by histology to confirm the presence of multiple tissues from each lineage (see *Figure 4*).

## Flow Cytometric Analysis of Pluripotent hES Cells

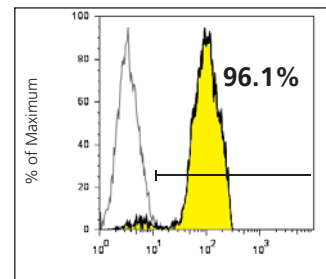
Oct-3/4



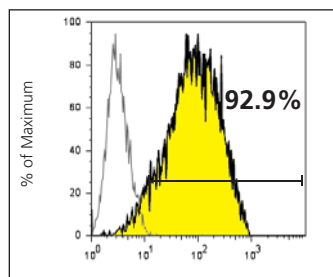
SSEA-3



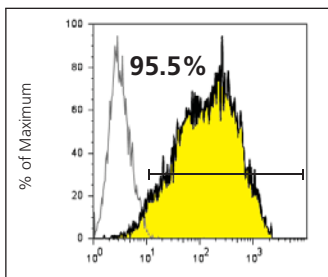
SSEA-4



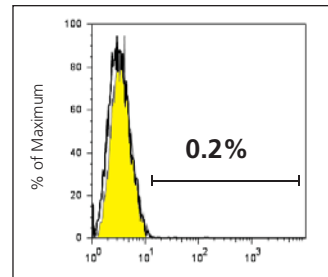
TRA-1-60



TRA-1-81

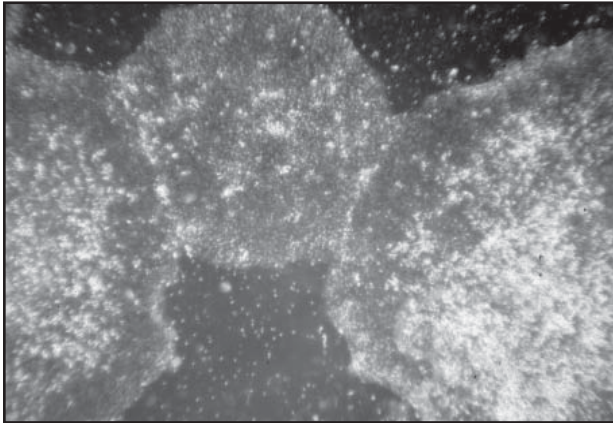


SSEA-1



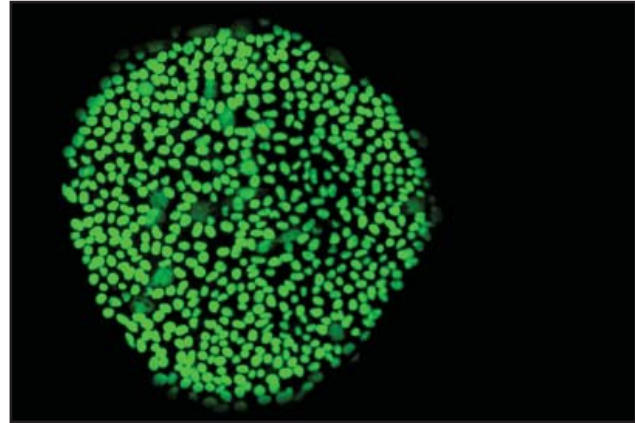
*Figure 1.* H9 cells (passage 48) grown on feeders in standard hES cell medium were transitioned to mTeSR™1 at the time of routine passaging. Cells were maintained in mTeSR™1 on BD Matrigel™ Matrix-coated dishes for 16 passages, with daily medium change and passaging every 6 days. At the end of 16 passages, cells were harvested for flow cytometric analysis of pluripotency markers using a BD FACSCalibur™ Flow Cytometer. H9 cells cultured in mTeSR™1 were found to be strongly positive for the cell surface carbohydrate SSEA-3 and the transcription factor Oct-3/4. Cells were > 92% positive for SSEA-4, TRA-1-60, and TRA-1-81, and negative for the differentiation marker SSEA-1.

## Morphology



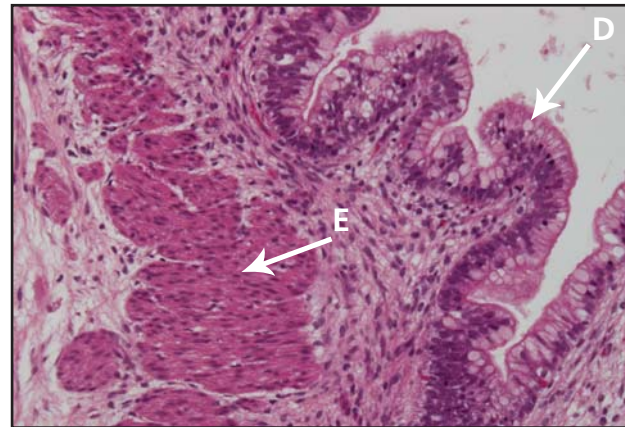
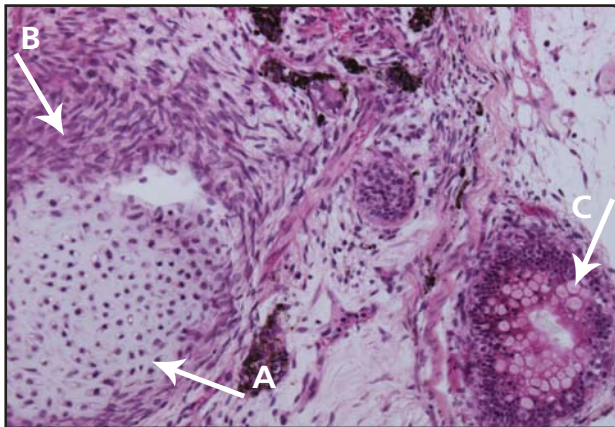
**Figure 2.** H9 cells cultured in mTeSR™1 for 10 passages (passage 43 total). hES cells grown in mTeSR™1 exhibit standard morphological hallmarks of the undifferentiated state: compact, multicellular colonies of cells with a high nucleus-to-cytoplasm ratio, prominent nucleoli, and a distinct colony border.

## Immunostaining



**Figure 3.** Immunohistochemical staining of a hES cell colony grown in mTeSR™1. A mouse monoclonal Oct-3/4 antibody and an anti-mouse FITC-conjugated secondary antibody were used.

## Teratoma Assay



**Figure 4.** Pluripotency of human ES cells maintained in mTeSR™1. H9 cells cultured in mTeSR™1 Medium on BD Matrigel™ Matrix for 5 passages formed teratomas when injected into NOD-SCID mice. These tumors were comprised of tissues from all three germ layers. Representative sections stained with hematoxylin and eosin contain: A. cartilage; B. mesenchymal-derived tissue; C. mucosal epithelium; D. gut-like structure; E. muscle.

## References

1. Ludwig T.E., et al., Feeder-independent culture of human embryonic stem cells, *Nature Methods* 3 (8):637-46 (2006).
2. Thomson, J.A., et al., Embryonic stem cell lines derived from human blastocysts, *Science* 282:1145 (1998).
3. Xu, C., et al., Feeder-free growth of undifferentiated human embryonic stem cells, *Nature Biotechnology* 19:971-4 (2001).
4. Xu, C., et al., Immortalized fibroblast-like cells derived from human embryonic stem cells support undifferentiated cell growth, *Stem Cells* 22:972-80 (2004).
5. Ludwig, T.E., et al., Derivation of human embryonic stem cells in defined conditions, *Nature Biotechnology*, 24:185-7 (2006).
6. Amit M., et al., Clonally derived human embryonic stem cell lines maintain pluripotency and proliferative potential for prolonged periods of culture, *Dev Biol.* 227:271-8, (2000).
7. Draper, J.S., et al., Surface antigens of human embryonic stem cells: changes upon differentiation in culture, *Journal of Anatomy*, 200:249-258.

# ORDERING INFORMATION

Description	Qty.	Cat. No.
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## StemCell Technologies Inc.

mTeSR™1 Maintenance Medium for Human Embryonic Stem Cells	500 mL (1 kit)	<b>05850</b>
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Please visit [www.stemcell.com](http://www.stemcell.com) for more information.

## BD Biosciences

BD Matrigel™ hESC-qualified Matrix	5 mL	<b>354277</b>
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Please visit [www.bdbiosciences.com/stemcellsource](http://www.bdbiosciences.com/stemcellsource) for more information.

## Coming Soon!

StemCell Technologies and BD Biosciences are collaborating on a defined, animal component-free cell culture environment for the maintenance of hES cells under feeder-independent conditions. Developed by researchers at WiCell Research Institute, TeSR™1 has been developed to be used with either defined extracellular matrices or BD Matrigel™ hESC-qualified Matrix<sup>5</sup>.

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