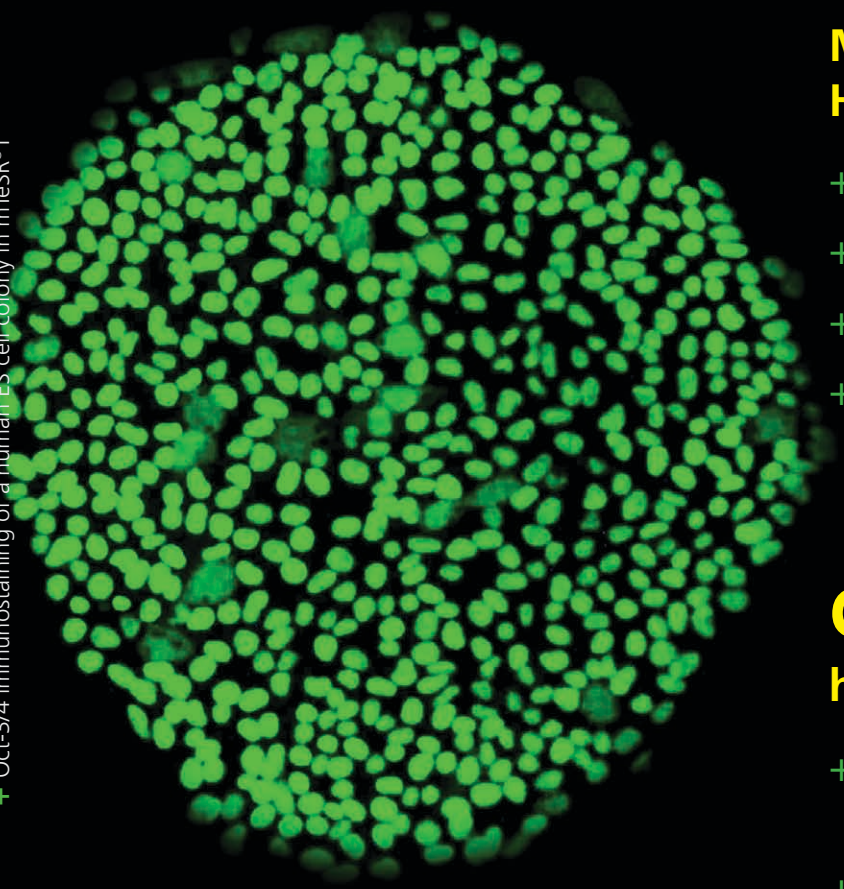
HUMAN EMBRYONIC STEM CELL
Culture Environments

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



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

mTeSR®1
Maintenance Medium for Human Embryonic Stem Cells

+ Feeder-Independent
+ WiCell™ Formulation[1]
+ Serum-Free
+ Defined

Corning® Matrigel®
hESC-qualified Matrix

+ Qualified as mTeSR1-Compatible
+ No Pre-Screening Required

StemCell Technologies Inc
The Cell Experts™

WiCell™ Research Institute

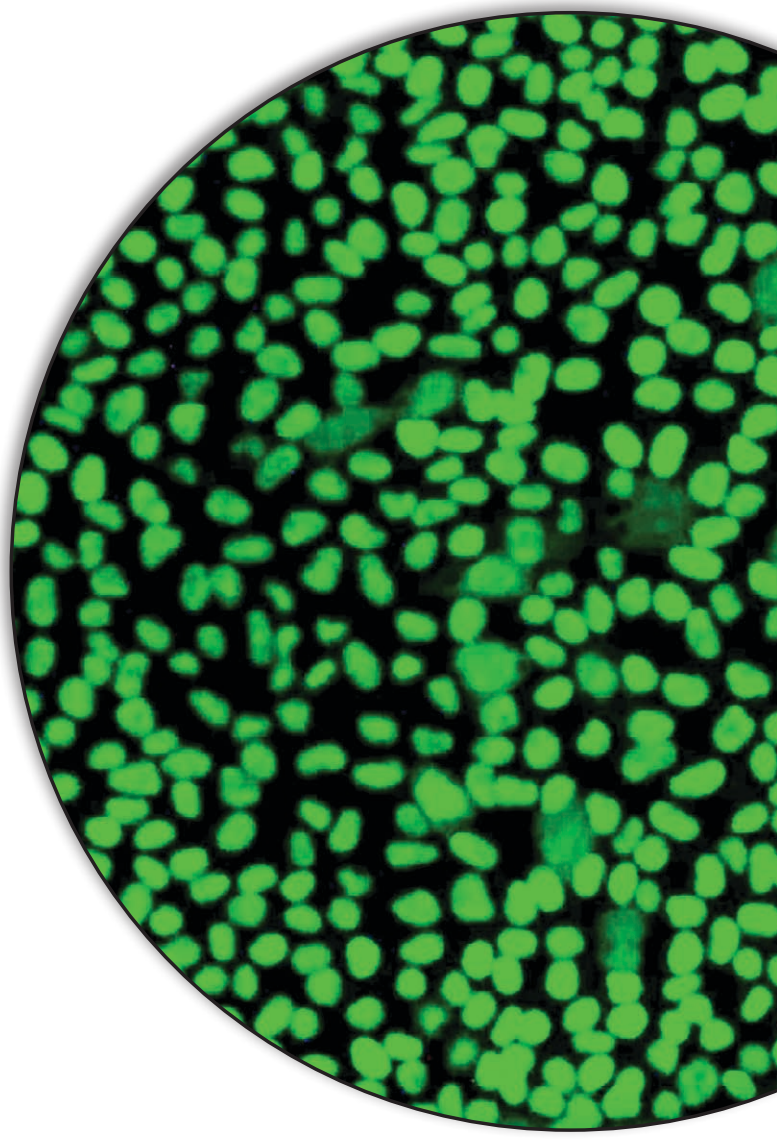
CORNING

HUMAN EMBRYONIC STEM CELL
Culture Environments

LEVERAGING EXPERTISE
to Advance Human Embryonic Stem 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. STEMCELL Technologies offers a number of products that 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[2]. This has lead 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[1] to standardize feeder-independent hES cell culture. mTeSR1 is complete, defined and serum-free, and has been designed to maintain and expand hES cells in an undifferentiated state when used with Corning® Matrigel® Matrix as a substrate. It does not require any further addition of growth factors. Undifferentiated hES cells maintained in mTeSR1 express high levels of pluripotency markers such as Oct-3/4 and SSEA-3. Pluripotency of cells maintained in mTeSR1 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. Corning has developed an optimized surface for your hES cell research. Corning Matrigel Matrix, coupled with a variety of culture media, has been widely accepted as an alternative substrate to feeder-dependent culture of hES cells[3,4]. Corning 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.

Corning now offers Corning Matrigel hESC-qualified Matrix for use with mTeSR1. Each batch has been qualified as mTeSR1-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 mTeSR1 formulation and Corning Matrigel Matrix have been shown to be a successful combination for culturing different WiCell™ hES cell lines for up to 20 passages[5].

mTeSR1 and Corning 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:*

mTeSR1

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

Corning Matrigel

hESC-qualified Matrix

+ Optimized surface for hES cell culture
+ Qualified as mTeSR1-compatible by StemCell Technologies

Benefits of mTeSR1 and Corning 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[7]. 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[1,6]. 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

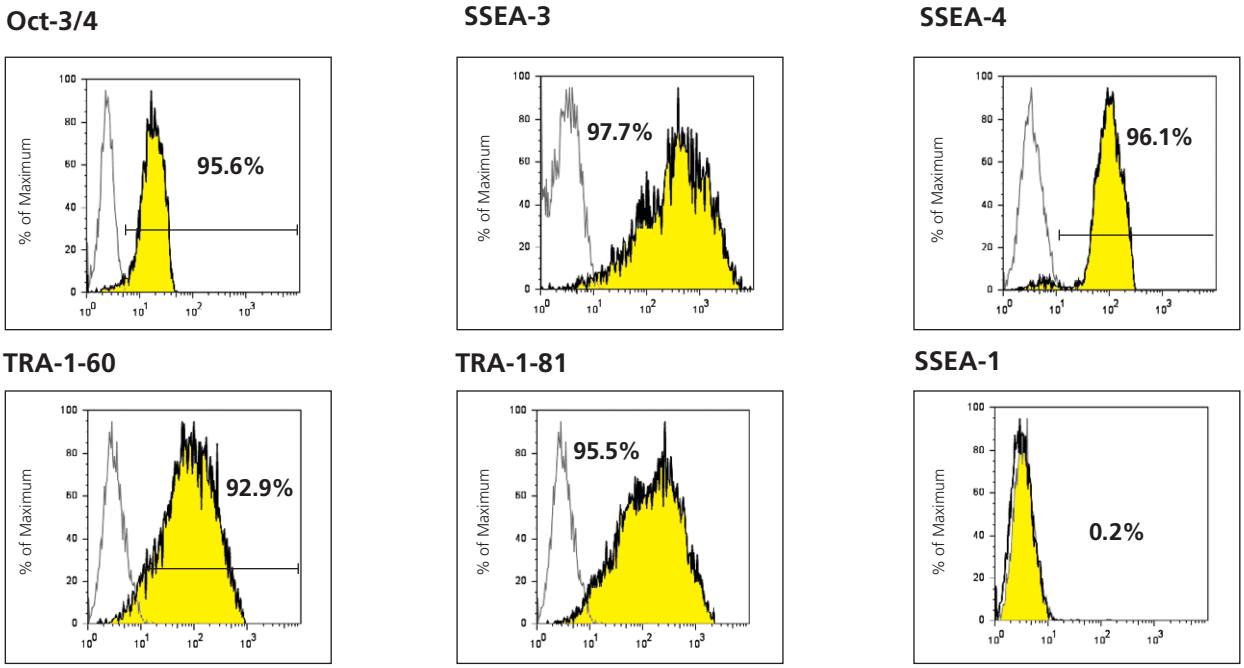


Figure 1. H9 cells (passage 48) grown on feeders in standard hES cell medium were transitioned to mTeSR®1 at the time of routine passaging. Cells were maintained in mTeSR1 on Corning® Matrigel® Matrix-coated dishes for 16 passages, with daily medium change and passaging every 6 days. At the end of 16 passages, cells were harvested for flow cytometric analysis of pluripotency markers using a BD FACSCalibur™ Flow Cytometer. H9 cells cultured in mTeSR1 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

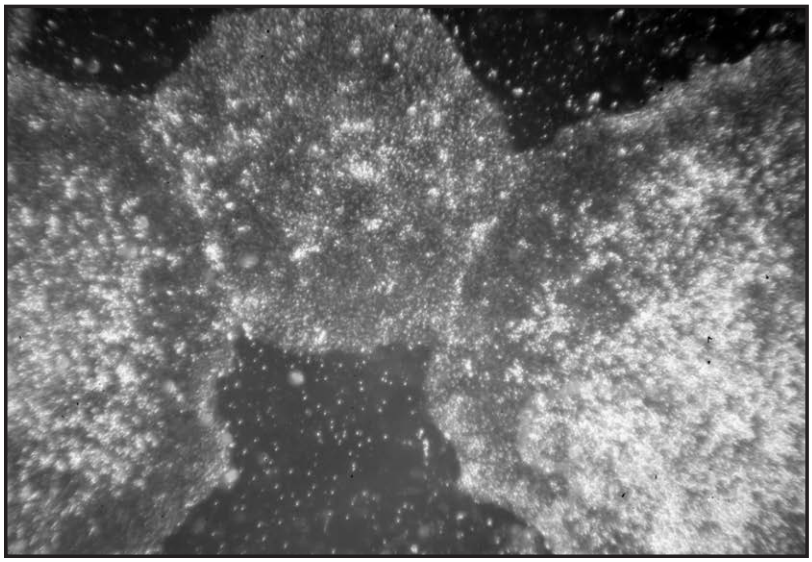


Figure 2. H9 cells cultured in mTeSR®1 for 10 passages (passage 43 total). hES cells grown in mTeSR1 exhibit standard morphological hallmarks of the undifferentiated state: compact, multicellular colonies of cells with a high nucleus-to-cytoplasm ratio, prominent nucleoli, and a distinct colony border.

Immunostaining

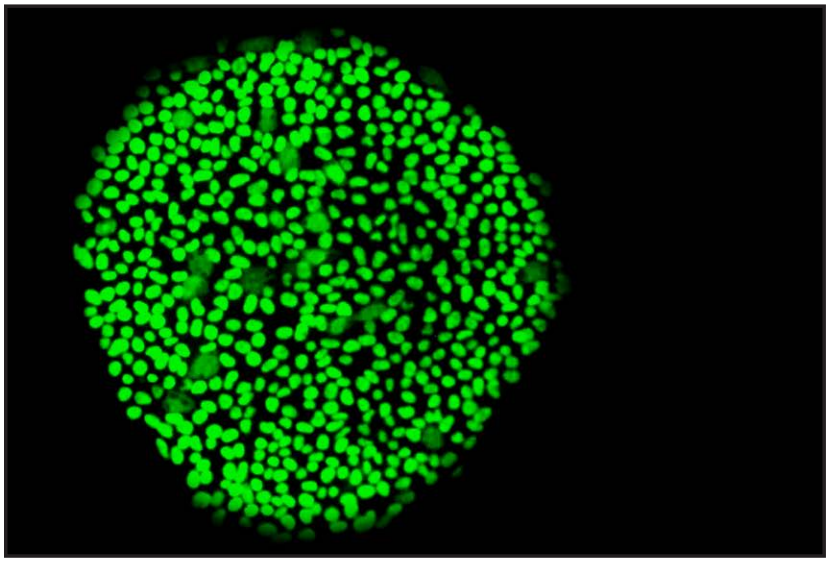


Figure 3. Immunohistochemical staining of a hES cell colony grown in mTeSR1. A mouse monoclonal Oct-3/4 antibody and an anti-mouse FITC-conjugated secondary antibody were used.

Teratoma Assay

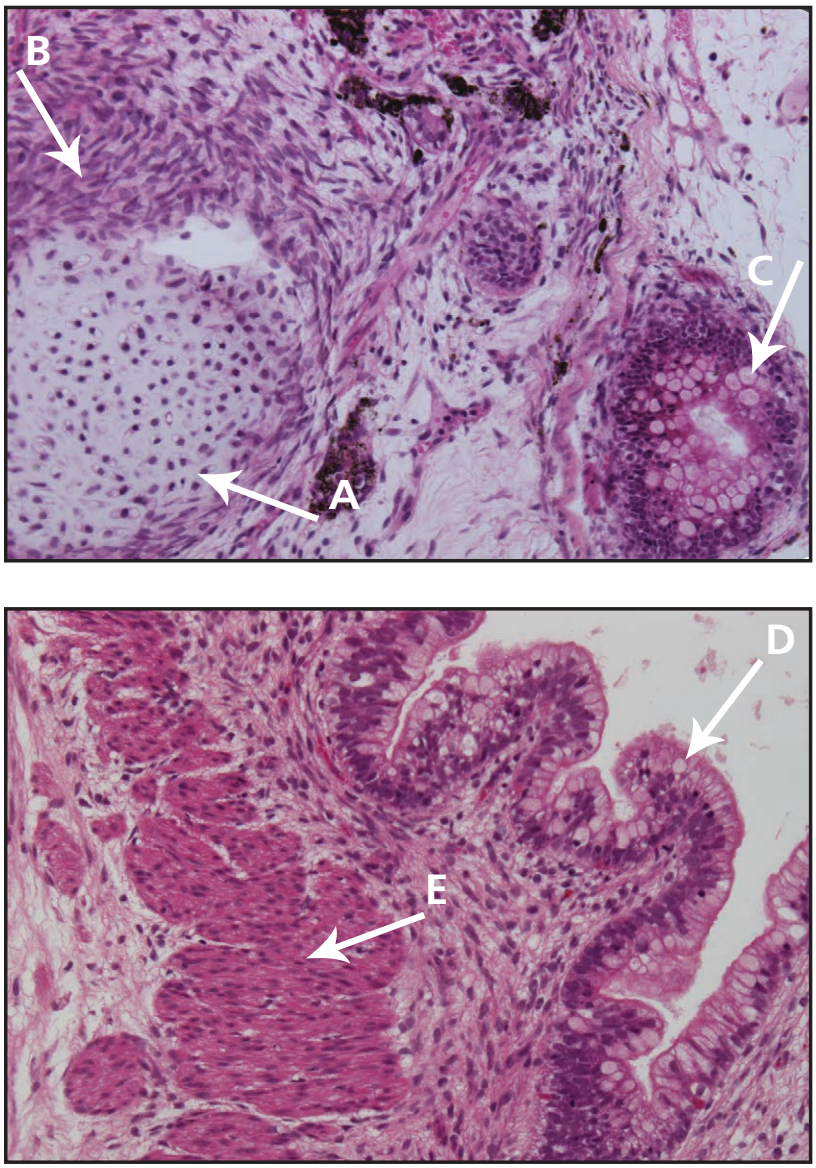


Figure 4. Pluripotency of human ES cells maintained in mTeSR1. H9 cells cultured in mTeSR1 Medium on Corning Matrigel® Matrix for 5 passages formed teratomas when injected into NOD-SCID mice. These tumors were comprised of tissues from all three germ layers. Representative sections stained with hematoxylin and eosin contain: **A.** cartilage; **B.** mesenchymal-derived tissue; **C.** mucosal epithelium; **D.** gut-like structure; **E.** muscle.

References

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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.
STEMCELL Technologies Inc.		
mTeSR®1 Maintenance Medium for Human Embryonic Stem Cells	500 mL (1 kit)	**05850**
Please visit **www.stemcell.com** for more information.		
Corning		
Corning® Matrigel® hESC-qualified Matrix	5 mL	**354277**
Please visit **www.corning.com/lifesciences** for more information		

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Developed by researchers at WiCell Research Institute, mTeSR1 has been developed to be used with either defined extracellular matrices or Corning Matrigel hESC-qualified Matrix[5].

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