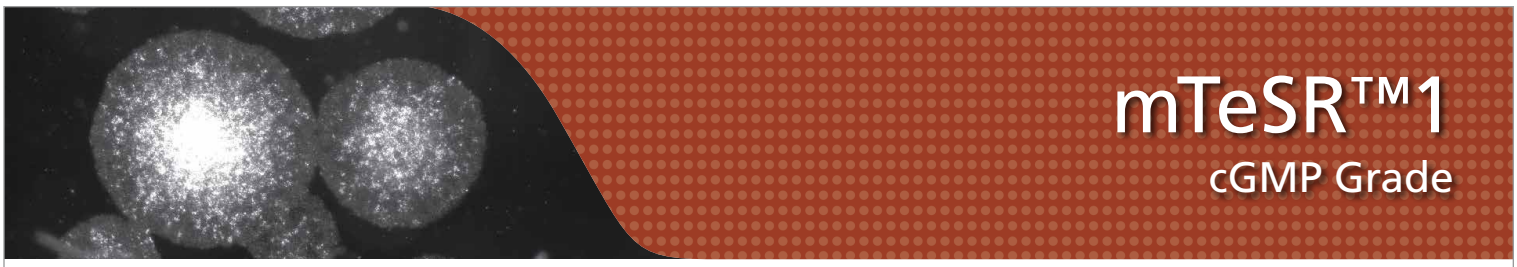

mTeSR™1
cGMP Grade

The Most Published Feeder-Free Culture Medium

mTeSR™1 is a highly specialized and defined, serum-free and complete cell culture medium, with established protocols for applications ranging from gene editing, bioreactor expansion, to lineage-specific differentiation. Proven to provide more consistent cultures with homogeneous, undifferentiated phenotypes, mTeSR™1 has been used to successfully maintain thousands of human pluripotent stem cell (hPSC) lines, enabling top hPSC research.

mTeSR™1 is known as the most reliable and consistent medium for hPSC culture. To ensure high levels of consistency in quality and performance from batch-to-batch, rigorous quality control testing and raw material screening is an integral part of the manufacturing process. In addition to a robust quality management system certified to ISO 13485:2003 standards, cGMP-grade mTeSR™1 is manufactured at an FDA-registered facility under a cGMP quality management system compliant to 21 CFR 820, under scalable cGMP conditions. Moreover, this culture medium is manufactured following the recommendations of USP <1043> on ancillary materials for cell, gene and tissue-engineered products, and is designed for use in cell therapy research applications and available for use under an approved investigational new drug (IND) application.

Developed at the:

WiCell® Research Institute

Advantages:

PROVEN. The most highly validated defined hPSC medium, backed by a decade of data. mTeSR™1 has been used in over 1500 peer-reviewed publications and is the medium of choice for feeder-free hPSC genome editing using CRISPR/Cas9.

FLEXIBLE. Supports multiple feeding and passaging timelines to suit your own schedule. mTeSR™1 can be combined with your cell culture matrix and passaging reagent of choice.

ROBUST. Contains pre-screened and quality-controlled components.

VERSATILE. Compatible with a peerless array of specialized reagents designed to support all traditional and cutting-edge applications.

cGMP. Ensures the highest quality and consistency for reproducible results.

Flexible Expansion and Maintenance of hPSC Culture

In addition to reliable and consistent performance, mTeSR™1 provides flexibility in the expansion and maintenance of hPSC culture, through versatile matrix and passaging reagent options, customizable passaging schedule, and convenient weekend-free protocol.

Successful culture of hPSCs requires a suitable matrix to allow attachment of cell aggregates. mTeSR™1 has been optimized for use with Vitronectin XF™, CellAdhere™ Laminin-521 or Corning® Matrigel®. Vitronectin XF™ and CellAdhere™ Laminin-521 are defined and xeno-free matrices to support the growth and differentiation of hPSCs. They are effective alternatives to Corning® Matrigel®.

hPSCs maintained in mTeSR™1 can be passaged using a number of different methods, both non-enzymatic and enzymatic. ReLeSR™ or Gentle Cell Dissociation Reagent are recommended for enzyme-free dissociation and passaging, and Dispase for enzymatic dissociation. ReLeSR™ and Gentle Cell Dissociation Reagent are both chemically-defined, animal component-free

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formulations. Passaging hPSCs with ReLeSR™ enables the easy generation of optimally-sized aggregates, while eliminating the hassle and variability associated with manual selection and scraping. Gentle Cell Dissociation Reagent is suitable for the dissociation of hPSCs into cell aggregates for routine passaging or into single-cell suspension.

Culturing hPSCs in mTeSR™1 allows flexibility in the passaging schedule, as cultures can be passaged between 4 and 7 days after plating. The frequency of passaging can be adjusted depending on the cell aggregate size and plating density. For example, if large cell aggregates are plated at a high density, the next passaging time will most likely occur on day 4 or 5, whereas if small cell aggregates are plated at a low density, the next passaging will most likely occur on day 6 or 7.

For more information, refer to the Technical Manual: Maintenance of Human Pluripotent Stem Cells in mTeSR™1 (Document #29106) available at **www.stemcell.com** or contact us to request a copy.

Weekend-Free Culture of hPSCs

mTeSR™1 and TeSR™-E8™ are robust media that can support a reduced feeding schedule, for example, eliminating the feeding of cultures on the weekend. In one suggested schedule (below), cells are only passaged once a week on Fridays, and medium replacement is not required on either Saturdays or Sundays. This protocol has been rigorously tested for routine use in long-term passaging. For more information and to see the data, refer to the Technical Bulletin: Weekend-Free Culture of Human Pluripotent Stem Cells in mTeSR™1 or TeSR™-E8™ (Document #28071) available at **www.stemcell.com** or contact us to request a copy.

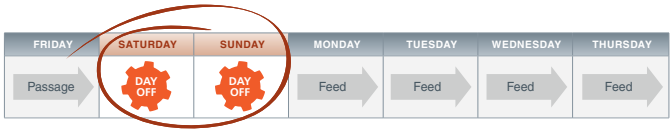

Figure 1. Example of Weekend-Free Schedule

By following a routine 7-day passaging schedule on Fridays, weekend work is eliminated as medium replacement is not required on either Saturdays or Sundays.

Genome Editing

Increase the Cloning Efficiency with CloneR™

Genome editing of hPSCs relies heavily on the survival of single cells to establish clonal lines. mTeSR™1 greatly enhances the cloning efficiency and single-cell survival of hPSCs when supplemented with **CloneR™**, especially under clonal and low-density seeding conditions. CloneR™ is a defined, serum-free supplement designed for use in feeder-free culture systems. Unlike current methods, CloneR™ enables the robust generation of clonal hPSC lines without single-cell adaptation, thus minimizing the risk of acquiring genetic abnormalities.

mTeSR™1 Supports Long-Term Maintenance of High-Quality hPSC Cultures

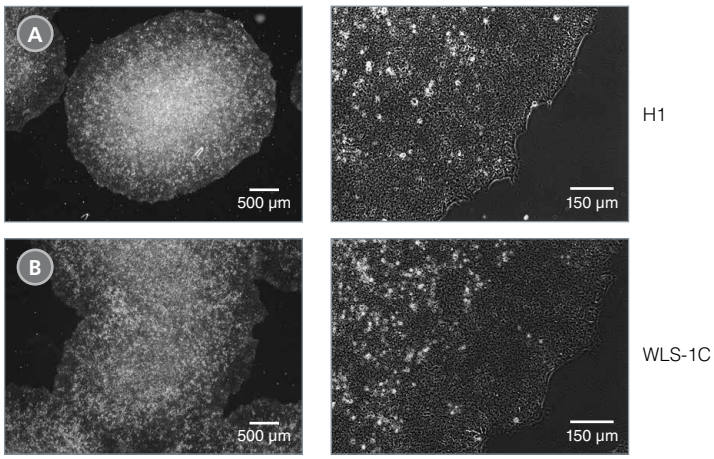

Figure 2. Normal hES and hiPS Cell Morphology is Observed in cGMP mTeSR™1 Cultures

Undifferentiated (A) H1 human embryonic stem (hES) and (B) WLS-1C human induced pluripotent stem (hiPS) cells cultured on Corning® Matrigel® Matrix in cGMP mTeSR™1 retain the prominent nucleoli and high nuclear-to-cytoplasmic ratio characteristic of this cell type after 10 passages. Densely packed cells and multi-layering are evident when cells are ready to be passaged.

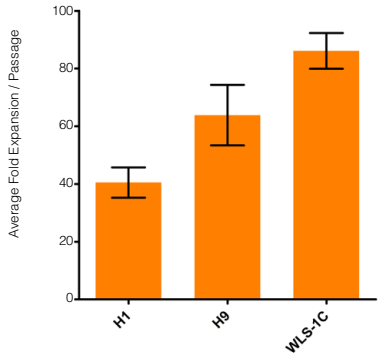

Figure 3. High Expansion Rates are Observed in cGMP mTeSR™1 Cultures

Graph shows the average fold expansion per passage +/- SEM obtained for hES (H1 and H9) and hiPS (WLS-1C) cells cultured in cGMP mTeSR™1 on Corning® Matrigel® Matrix over 10 passages. Expansion was determined by enumerating the cell aggregates obtained at harvest and dividing by the number of cell aggregates seeded. Note that this data is representative of cultures passaged after 6 - 7 days in culture, and lower expansion should be expected if using shorter culture times.

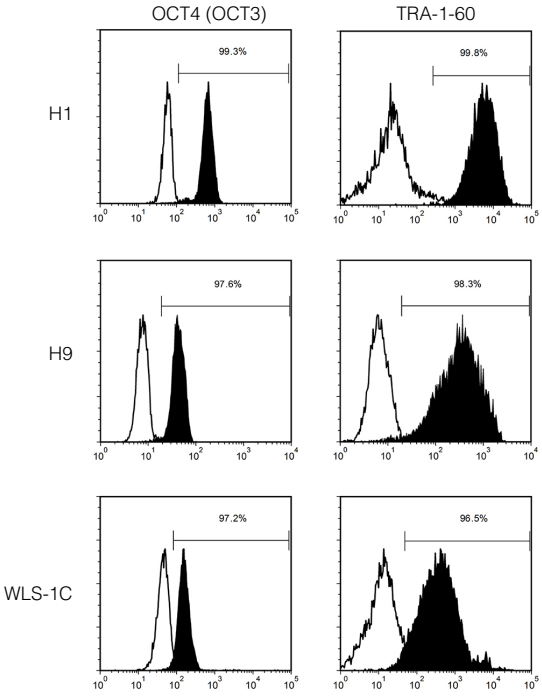

Figure 4. Cells Cultured in cGMP mTeSR™1 Medium Express Undifferentiated Cell Markers

(A) Histogram analysis for hES (H1 and H9) and hiPS (WLS-1C) cells characterized using flow cytometry for undifferentiated cell markers, OCT4 (OCT3) (Catalog #60093) and TRA-1-60 (Catalog #60064), after 8 - 10 passages in cGMP mTeSR™1 (filled = sample, blank = isotype control).

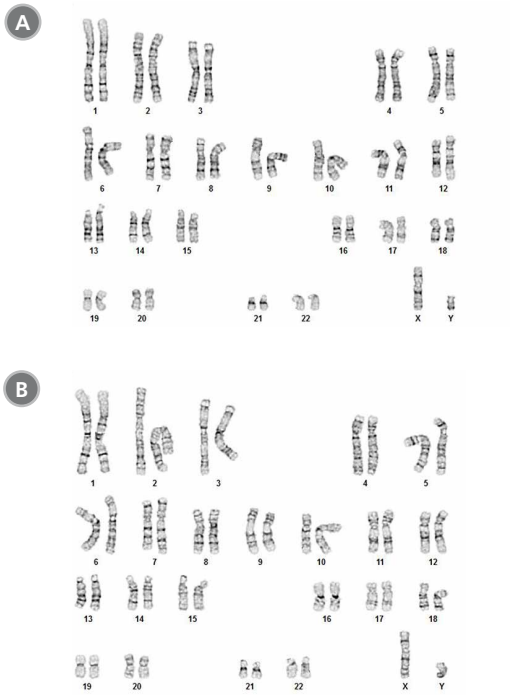

Figure 5. hPSCs Maintained in cGMP mTeSR™1 Display a Normal Karyotype

Karyograms of (A) H1 hES and (B) WLS-1C hiPS cells cultured in cGMP mTeSR™1 for 11 passages show that a normal karyotype is retained.

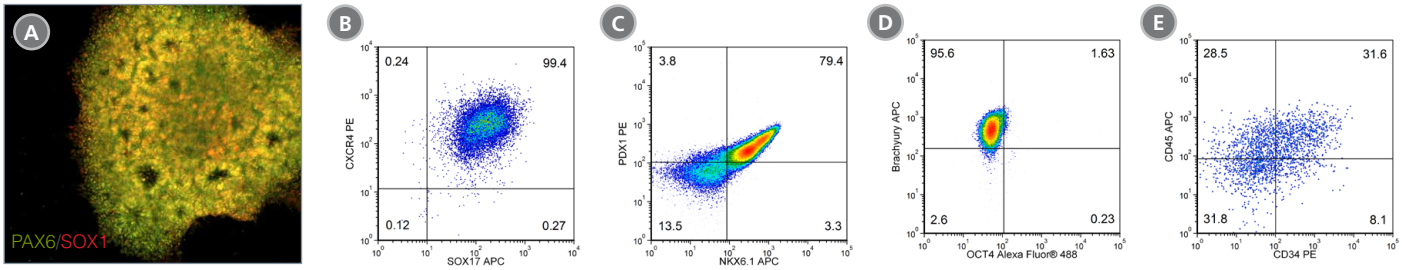

Figure 6. hPSCs Maintained in cGMP mTeSR™1 are Capable of Differentiation to All Three Germ Layers

Human ES cells (H9) maintained in cGMP mTeSR™1 were differentiated to ectoderm, endoderm, and mesoderm lineages. (A) Ectoderm specification was achieved using STEMdiff™ Neural Induction Medium (Catalog #05835). A representative image of resulting neural rosettes, expressing PAX6 (green) and SOX1 (red), is shown. Endoderm specification was demonstrated by culturing cells in (B) STEMdiff™ Definitive Endoderm Kit (Catalog #05110) and (C) STEMdiff™ Pancreatic Progenitor Kit (Catalog #05120). Mesoderm specification was achieved using (D) STEMdiff™ Mesoderm Induction Medium (Catalog #05220) and (E) STEMdiff™ Hematopoietic Kit (Catalog #05310). Representative flow cytometry plots are shown.

cGMP mTeSR™1

Product Information

PRODUCT	SIZE	CATALOG #
mTeSR™1	500 mL	85850
	1 L	85857
	10 Kits	85870
	25 Kits	85875

Related Products

PRODUCT	SIZE	CATALOG #
CloneR™	10 mL	05888
	5 x 10 mL	05889
Vitronectin XF™ Kit*	1 Kit	07190
Vitronectin XF™	2 mL	07180
CellAdhere™ Laminin-521	100 µg	77003
	1 mg	77004
ReLeSR™	100 mL	05872
	500 mL	05873
Gentle Cell Dissociation Reagent	100 mL	07174
Dispase	1 U/mL	07923

*Kit contains Vitronectin XF™, CellAdhere™ Dilution Buffer, Gentle Cell Dissociation Reagent and Non-Tissue-Culture-Treated 6-Well Plates.

Accessory Products

Cryopreservation Media

PRODUCT	SIZE	CATALOG #
mFreSR™	50 mL	05855
	10 x 5 mL Tubes	05854
FreSR™-S	50 mL	05859
CryoStor® CS10	100 mL	07930
	5 x 16 mL Vials	07931
	1000 mL Bag	07940
	100 mL Bag	07955
	5 x 10 mL Vials	07959
	6 x 10 mL Syringes	07942

For a complete list of related products, including specialized cell culture and storage media, matrices, antibodies, cytokines and small molecules, visit **www.stemcell.com/hPSCworkflow** or contact us at **techsupport@stemcell.com**.

Reference

1. Ludwig TE et al. (2006) Nat Methods 3(8): 637-46.