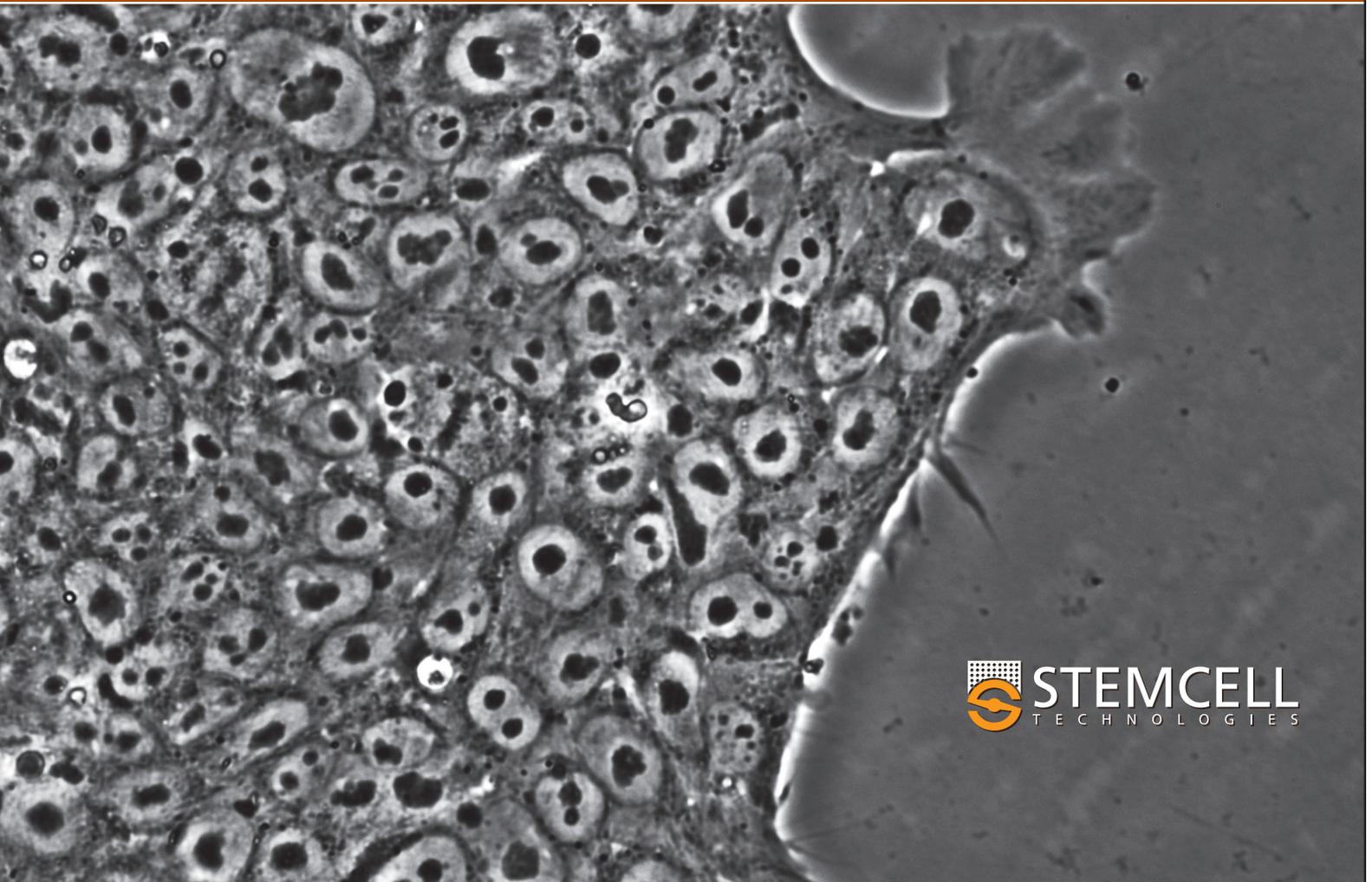


PRODUCTS FOR HUMAN
PLURIPOTENT STEM CELLS

STANDARDIZED SOLUTIONS FOR
hESC & hiPSC RESEARCH



 **STEMCELL**
TECHNOLOGIES

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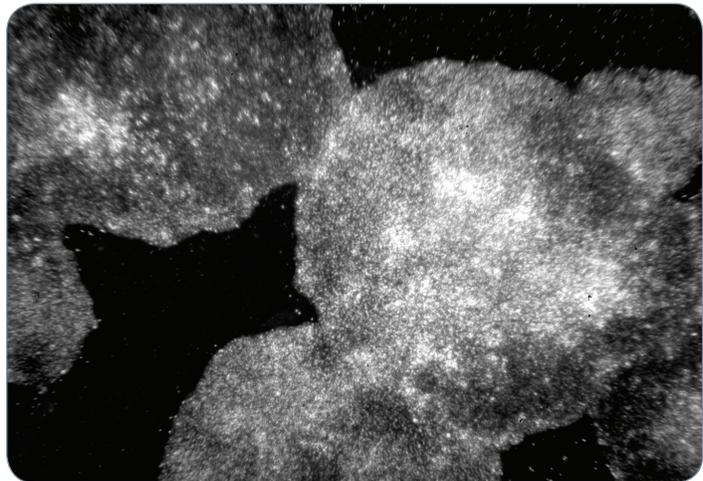
Standardizing human pluripotent stem cell culture.

STEMCELL Technologies is pleased to provide a set of standardized tools and solutions designed to help your research with human embryonic and induced pluripotent stem cells.

PRODUCTS FOR HUMAN **PLURIPOTENT STEM CELL** RESEARCH

INTRODUCTION

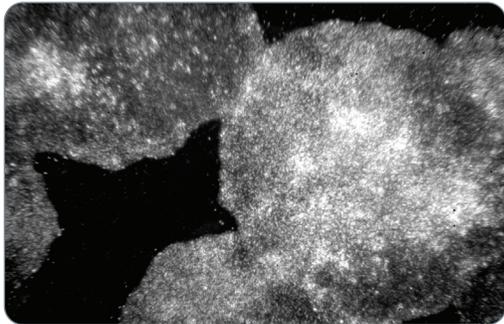
Undifferentiated human pluripotent stem cells have the potential for unlimited expansion with the retention of normal karyotype and the ability to generate cells of all three germ layers — endoderm, mesoderm and ectoderm.¹⁻⁴ These germ layer cells can then further differentiate into many specific cell lineages.⁵ Because of this ability, their use has been proposed in a variety of clinical applications and as a tool for the study of human cellular and developmental systems. Human cells with pluripotent characteristics were initially derived from the inner cell mass of pre-implantation blastocysts and termed human embryonic stem cells (hESCs).⁵ The recent discovery that human fibroblasts²⁻⁴ can be reprogrammed by the transient overexpression of a small number of genes into induced pluripotent stem cells (iPSCs), which functionally and phenotypically resemble hESCs, raises the possibility that cellular therapies using patient-specific input cells may be a reality in the future.



PRODUCTS FOR HUMAN PLURIPOTENT STEM CELL RESEARCH

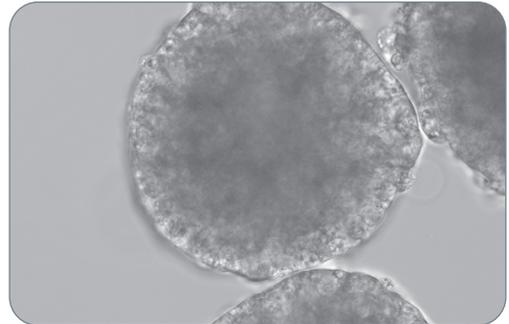
STEMCELL Technologies provides a full range of products to expand and characterize undifferentiated and differentiated cells.

MAINTENANCE



Undifferentiated hESCs

EMBRYOID BODY FORMATION



Embryoid bodies

PRODUCT	QUANTITY	CATALOG #
MAINTENANCE MEDIA		
mTeSR®1	500 mL	05850
	10 x 500 mL	05870
	25 x 500 mL	05875
TeSR™2	500 mL	05860
	10 x 500 mL	05880
CRYOPRESERVATION MEDIA		
mFreSR®1	10 x 5 mL	05854
	50 mL	05855
CryoStor™CS10	100 mL	07930

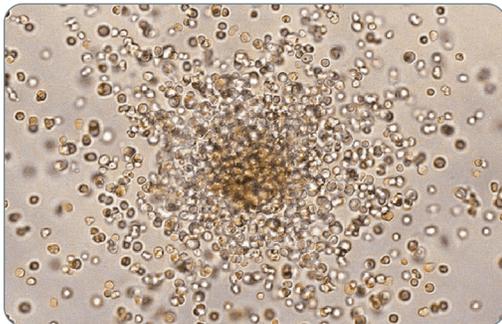
NEW!

NEW!

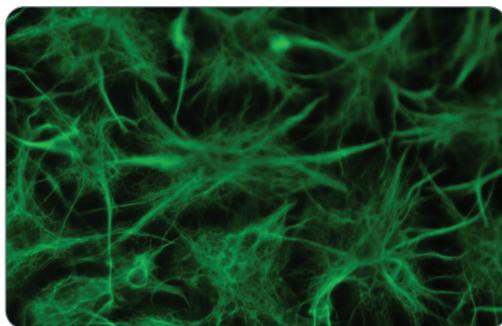
PRODUCT	QUANTITY	CATALOG #
EMBRYOID BODY FORMATION		
AggreWell™400 plate 8 wells, with approximately 1,200 microwells per well	1/pack	27845
	5/pack	27945
AggreWell™800 plate 8 wells, with approximately 300 microwells per well	1/pack	27865

COMING
SOON!

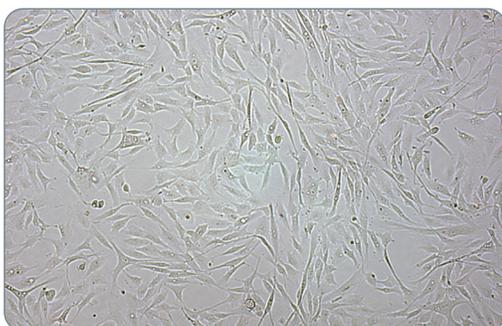
DIFFERENTIATION



CFU-GM colony in methylcellulose.



Immunofluorescent staining of astrocytes (green) identified with rabbit polyclonal anti-GFAP (Catalog #01415).



Confluent mesenchymal stem cells.

HEMATOPOIETIC CELLS

STEMCELL Technologies offers the following products for the isolation, culture and characterization of human hematopoietic progenitors:

- **EasySep® hESC-derived CD34 Positive Selection Kit** - optimized kit for the isolation of highly purified hESC-derived CD34⁺ cells.
- **MethoCult®** - methylcellulose-based media optimized for use in hematopoietic colony assays.
- **MyeloCult®** - media optimized for long-term culture of hematopoietic cells.
- **StemSpan®** - serum-free medium for the culture and expansion of hematopoietic stem and progenitor cells.

NEURAL CELLS

STEMCELL Technologies offers optimized, serum-free and animal component-free media for the culture of human neural stem cells. The **NeuroCult®** product line includes standardized reagents for the proliferation and differentiation of neural stem and progenitor cells.

MESENCHYMAL CELLS

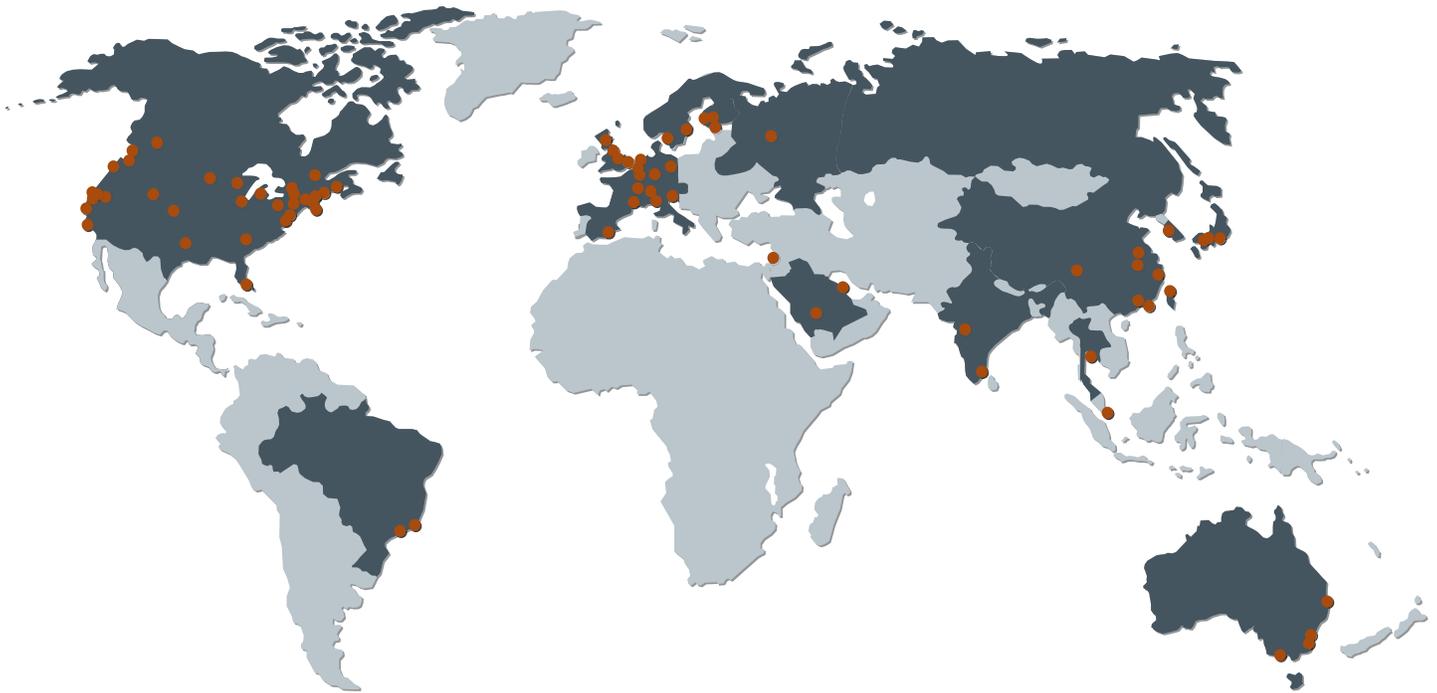
STEMCELL Technologies offers optimized products for the enrichment, expansion and differentiation of human mesenchymal stem cells:

- **MesenCult®-ACF Medium** - an animal component-free, serum-free culture medium for human MSCs that provides superior performance compared to traditional serum-containing formulations.
- **MesenCult® Proliferation Kits (Human)** - serum-containing medium optimized for cell expansion and CFU-F assays. Contains prescreened components that minimize lot-to-lot variability.

mTeSR[®]1

Development of serum and feeder-free culture conditions for human pluripotent stem cells

mTeSR[®]1 is a bovine serum albumin (BSA)-containing medium that supports the long-term, feeder-independent culture of hESCs⁶ and hiPSCs³ as well as the derivation of hiPSCs in feeder-independent conditions.¹⁵ It was developed at the WiCell Research Institute and includes high levels of basic fibroblast growth factor (bFGF) together with transforming growth factor β (TGF β), γ -aminobutyric acid (GABA), pipercolic acid and lithium chloride. To date, mTeSR[®]1 has been shown to work on more than 50 independently derived hESC lines as well as hiPSCs.



mTeSR[®]1 is being used in more than 26 countries on more than 50 independently derived cell lines, including human induced pluripotent stem cells.

mTeSR[®]1

DEFINED, FEEDER-INDEPENDENT MAINTENANCE MEDIUM FOR HUMAN PLURIPOTENT STEM CELLS



STEMCELL Technologies has developed mTeSR[®]1 (Catalog #05850/05870/05875) as a standardized medium for feeder-independent maintenance of human pluripotent stem cells, specifically human embryonic stem cells (hESCs)⁶ and human induced pluripotent stem cells (hiPSCs).³ It is a complete, serum-free, defined formulation based on the publication by Ludwig *et al.*⁶ developed and manufactured under license from the WiCell Research Institute. The medium does not require any further addition of growth factors.

mTeSR[®]1 has been designed for use with BD Matrigel[™] hESC-qualified Matrix (BD Catalog #354277) as a substrate. STEMCELL Technologies has pre-qualified each batch of BD Matrigel[™] to ensure consistency, reproducibility and reliability in performance.

mTeSR[®]1 PROVIDES:

- Consistent maintenance conditions for hESC and hiPSC culture due to the elimination of undefined medium components and removal of the inherent variability associated with feeder cells and conditioned media
- Standardization of culture methods leading to increased reproducibility of data
- Time savings due to elimination of the labor involved in preparing feeder cells or conditioned media
- A complete medium formulation with no additional growth factors or other supplements necessary

PRODUCT	QUANTITY	CATALOG #
mTeSR [®] 1	500 mL	05850
	10 x 500 mL	05870
	25 x 500 mL	05875

NEW!

Interested in pre-clinical studies?

Ask us about mTeSR[®]1 that is manufactured in a GMP facility by contacting STEMCELL's Technical Support at technicalsupport@stemcell.com.

Developed at the:



mTeSR[®]1 Cultures Show Consistent Expansion

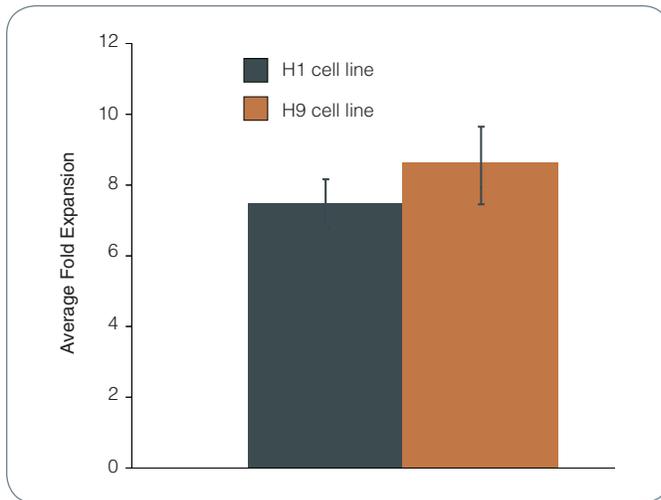
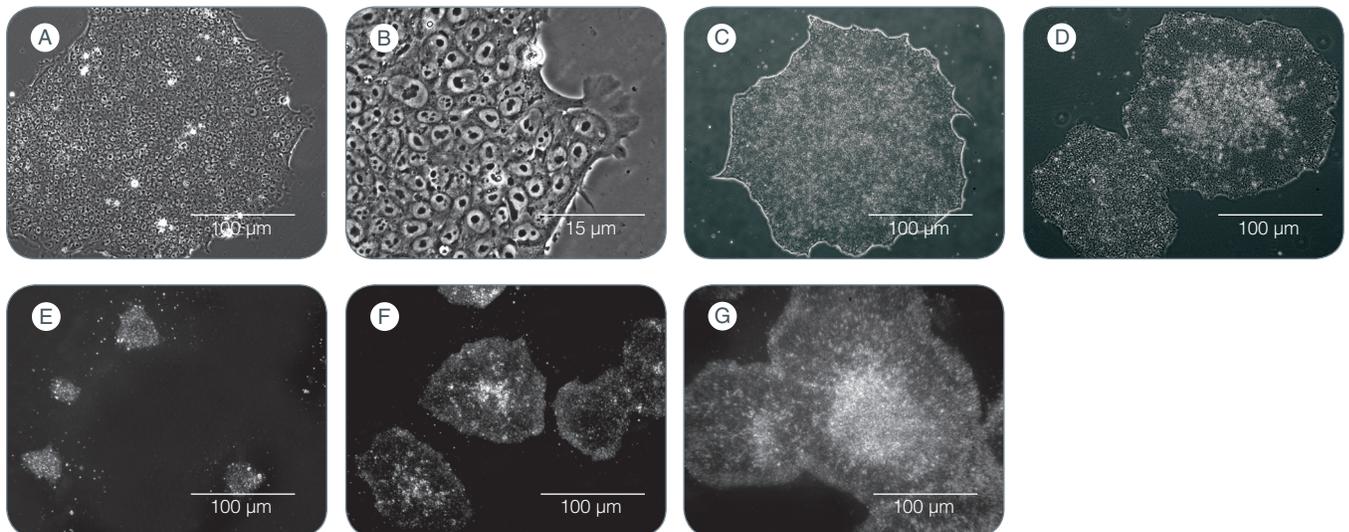


FIGURE 1: H1 and H9 hESCs were expanded in mTeSR[®]1 for 19 and 18 passages respectively. Cultures show consistent expansion at each passage.

Morphology of Human Embryonic and Induced Pluripotent Stem Cells Cultured in mTeSR[®]1



DAY 2: Colonies are small, transparent and contain few cells.

DAY 4: Colonies rapidly increase in size and start to develop phase-bright centers when viewed under phase contrast.

DAY 6: Colonies begin to merge and have phase-bright centers that are densely packed with cells. These colonies are ready to passage.

FIGURE 2: H1 hESCs grow as colonies with (A) defined edges and (B) high nucleus to cytoplasm ratio. hiPSC lines (C) iPSC(IMR90)-3 and (D) MSC-iPSC1 maintained in mTeSR[®]1 show similar morphological characteristics. (E-G) H9 hESCs are routinely passaged every 5 - 7 days. The morphology of hESCs in culture varies slightly compared to feeder-containing or conditioned medium cultures.

hiPSC photographs courtesy of M. O'Connor and C. Eaves, The Vancouver Human Embryonic Stem Cell Core Facility

mTeSR[®]1

KARYOTYPE & PLURIPOTENCY

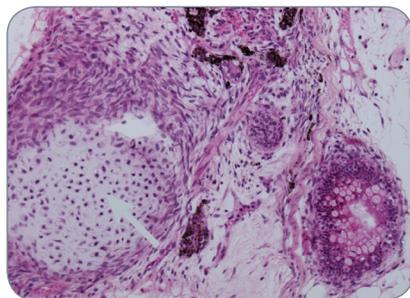
Human Embryonic Stem Cells Cultured in mTeSR[®]1 Retain Normal Karyotype Following Long-Term Passage



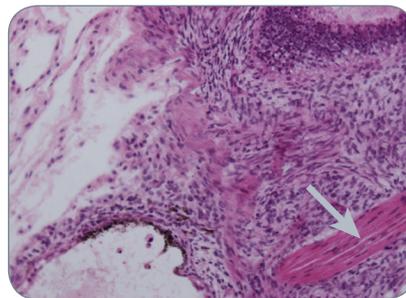
FIGURE 3: Chromosomal analysis of H1 hESCs cultured in mTeSR[®]1 for 48 passages shows that normal karyotype is retained during long-term passaging.

Data from Dr. T Ludwig, WiCell Research Institute

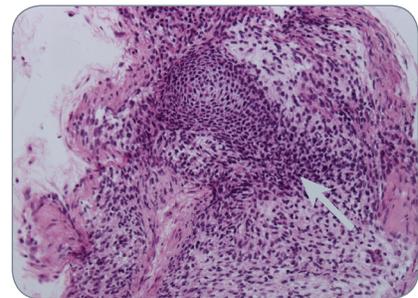
Human Embryonic Stem Cell Cultures in mTeSR[®]1 are Pluripotent



Cartilage



Muscle



Neural

FIGURE 4: 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.

TeSR™2

ANIMAL PROTEIN-FREE, DEFINED, FEEDER-INDEPENDENT MAINTENANCE MEDIUM FOR HUMAN PLURIPOTENT STEM CELLS



Given the interest in using hESCs and hiPSCs for applications in regenerative medicine, the development of fully humanized and defined systems for the maintenance of hESCs and hiPSCs is critical. TeSR™2 is a significant step in this direction.

TeSR™2 is a human albumin containing medium which allows hESCs and hiPSCs to be cultured feeder-free in animal protein-free, defined medium.¹⁴

PRODUCT	QUANTITY	CATALOG #
TeSR™2	500 mL	05860
	10 x 500 mL	05880

NEW!

ADVANTAGES OF TESR™2

- Animal protein-free, defined
- Reduced variability in hESC and hiPSC culture
- No need to grow feeders

EXPANSION & MORPHOLOGY

Expansion of hESCs Cultured in TeSR™2

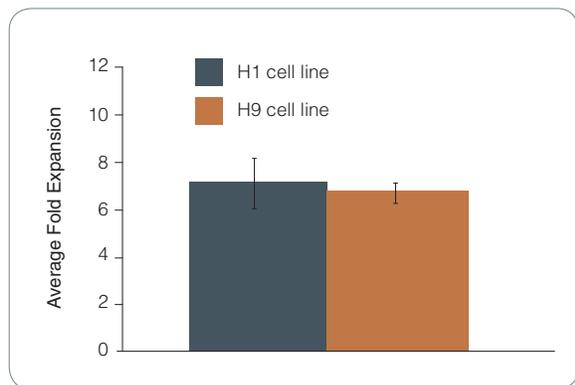


FIGURE 5: hESCs cultured in TeSR™2 demonstrate consistent expansion.

Morphology of hiPSCs Cultured in TeSR™2

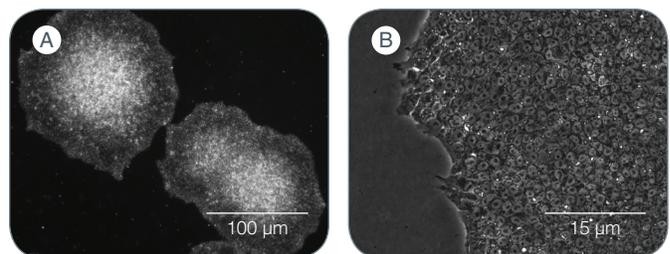


FIGURE 6: hiPSCs cultured in TeSR™2 grow as colonies with defined edges and high nucleus to cytoplasm ratio. iPS(IMR90)-1 cell line cultured (A) for 8 passages in TeSR™2 and (B) for 10 passages in TeSR™2.

hiPSC photographs courtesy of Dr. T. Ludwig, WiCell Research Institute

TeSR™2

KARYOTYPE & PLURIPOTENCY

hESCs Cultured Long-Term in TeSR™2 Retain Normal Karyotype

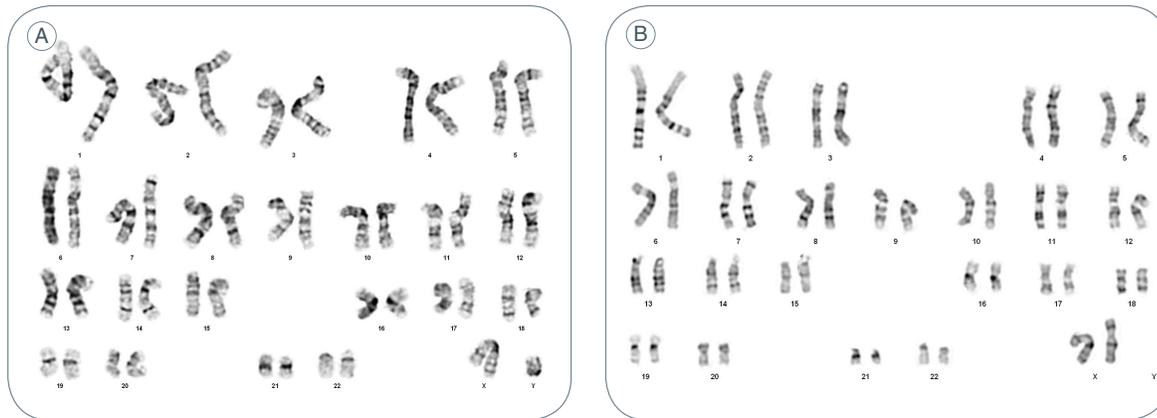


FIGURE 7: Retention of normal karyotype in (A) H1 and (B) H9 cells following long-term passaging in TeSR™2, 19 and 22 passages respectively.

hESCs Cultured in TeSR™2 are Pluripotent

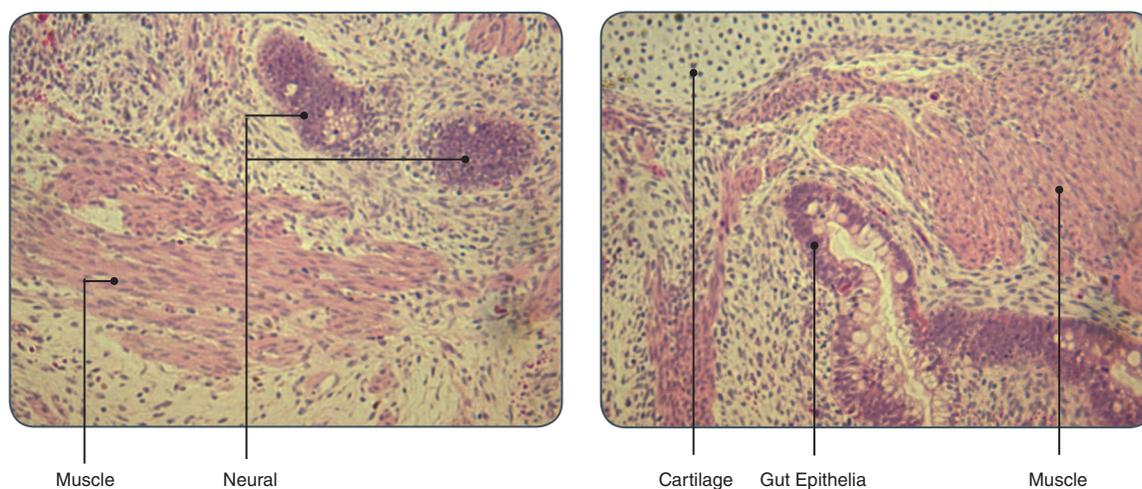


FIGURE 8: H9 cells were cultured for 11 passages in TeSR™2 then injected subcutaneously into NOD-SCID mice. The resulting teratomas contained cell types from all 3 germ layers. Representative tissue types are shown.

CHARACTERIZATION OF HUMAN PLURIPOTENT STEM CELLS

STEMCELL offers a wide range of primary and secondary antibodies suitable for the characterization of hESCs and hiPSCs during culture maintenance and expansion. Undifferentiated hESCs and hiPSCs express high levels of certain pluripotency markers, such as Oct-3/4 and SSEA-3, and whose detection can assist in determining the undifferentiated state of a particular hESC or hiPSC population.

Primary Antibodies

TARGET ANTIGEN	CLONE	ISOTYPE	CATALOG #	QUANTITY
Oct-3/4	40	Mouse IgG ₁	01550	50 µg
			01551	150 µg
SSEA-1	MC-480	Mouse IgM	01552	100 tests
SSEA-3	MC-631	Rat IgM	01553	100 tests
SSEA-4	813-70	Mouse IgG ₃	01554	100 tests
TRA-1-60	TRA-1-60	Mouse IgM	01555	100 tests
TRA-1-81	TRA-1-81	Mouse IgM	01556	100 tests
TRA-2-49	TRA-2-49/6E	Mouse IgG ₁	01557	100 tests
TRA-2-54	TRA-2-54/2J	Mouse IgG ₁	01558	100 tests

NEW!

Secondary Antibodies

TARGET ANTIGEN	HOST SPECIES	FORMAT	CATALOG #	QUANTITY	FOR USE WITH
Mouse IgG	Goat	FITC	10210	1.5 mg	Anti-TRA-2-49 Anti-TRA-2-54 Anti-SSEA-4 Anti-Oct-3/4
Mouse IgM	Goat	FITC	10211	1.5 mg	Anti-SSEA-1 Anti-TRA-1-60 Anti-TRA-1-81
Rat IgM	Goat	APC	10215	0.25 mg	Anti-SSEA-3

Immunohistochemistry of Human Embryonic Stem Cells Cultured in mTeSR®1

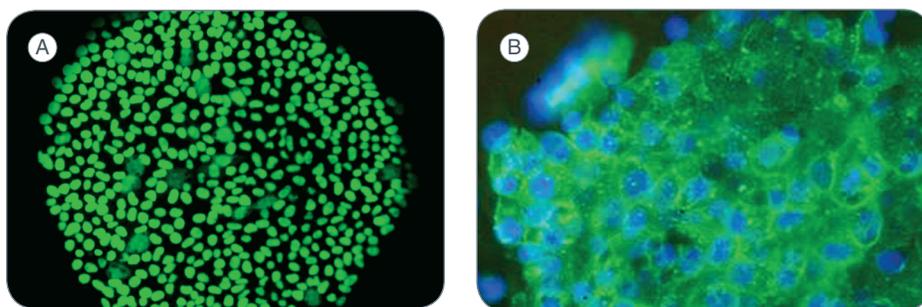


FIGURE 9: Immunohistochemistry of H1 hESCs cultured in mTeSR®1 reveal uniform expression of pluripotency markers throughout the colony (A) Oct-3/4 (Catalog #01550/01551) with FITC-conjugated secondary antibody (Catalog #10210). (B) SSEA-4 (Catalog #01554) with FITC-conjugated secondary antibody (Catalog #10210); DAPI staining shows nuclei.

Human Embryonic Stem Cells Cultured in either mTeSR®1 or TeSR™2 Express High Levels of Pluripotent Markers and Low Levels of Differentiation Markers

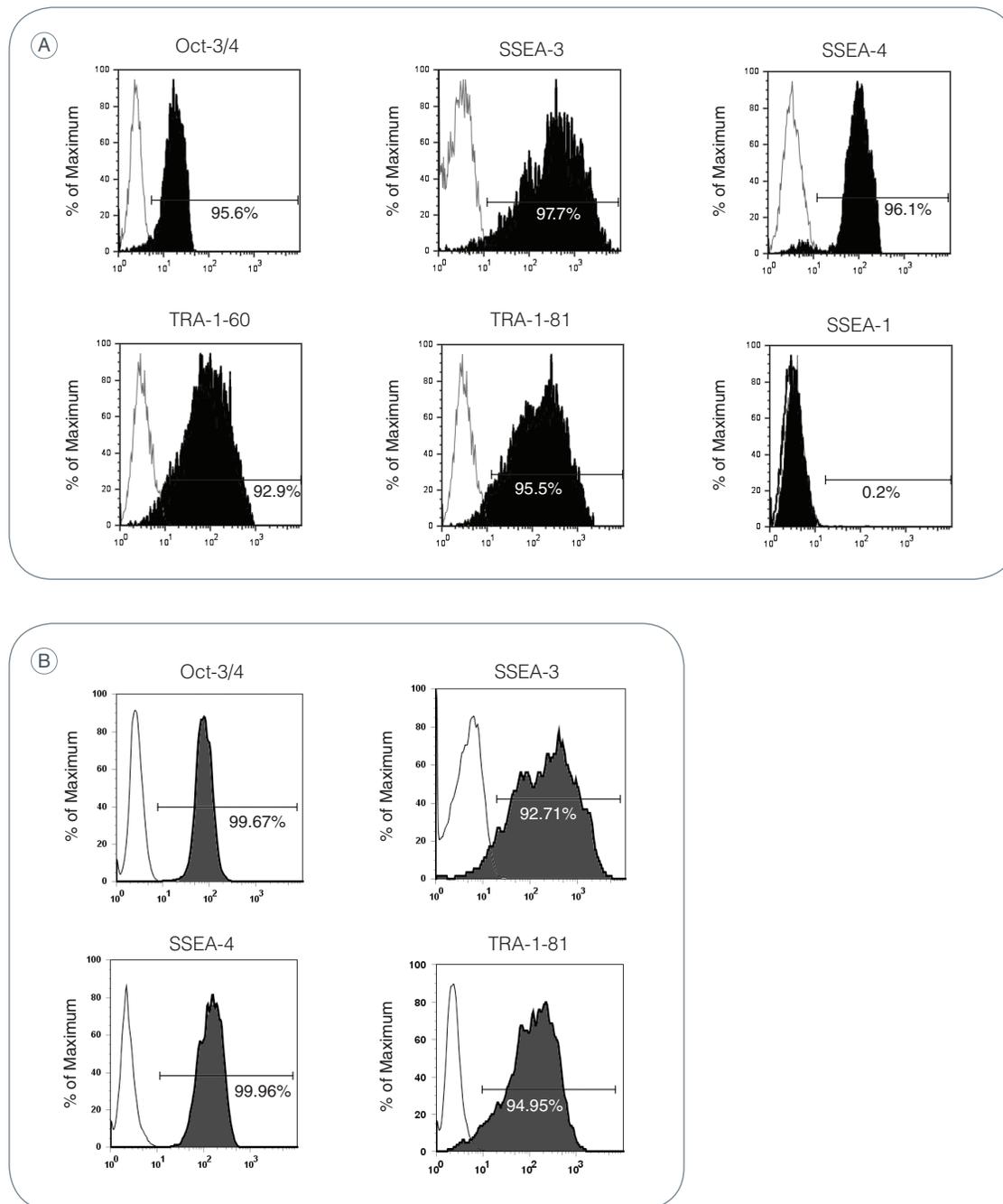


FIGURE 10: (A) Flow cytometric analysis of H9 hESCs maintained in mTeSR®1 for 17 passages. (B) Flow cytometric analysis of H1 cells maintained in TeSR™2 for 26 passages.

AggreWell™

REPRODUCIBLE, UNIFORM EMBRYOID BODIES



Many hESC and hiPSC differentiation protocols begin with the formation of 3-dimensional aggregates of cells called embryoid bodies (EBs).¹² Conventional EB formation methods involve scraping undifferentiated cell cultures to release large clumps of cells and placing the resulting aggregates in suspension culture. The EBs formed are heterogeneous in size and shape leading to inefficient and uncontrolled differentiation.

AggreWell™ plates contain microwells which can be used to aggregate cells into EBs by plating a single cell suspension and then culturing for 24 hours. The resulting EBs are highly uniform in size (Figure 11) and can be efficiently differentiated into a variety of cell types.¹³ AggreWell™ plates bring a standardized approach to the production of EBs making experiments more reproducible over time.

PRODUCT	QUANTITY	CATALOG #
AggreWell™400 plate 8 wells, with approximately 1,200 microwells per well	1/pack	27845
	5/pack	27945
AggreWell™800 plate 8 wells, with approximately 300 microwells per well	1/pack	27865

EMBRYOID BODIES GENERATED USING AGGREWELL™ PLATES ARE:

- Uniform in size and shape
- Reproducible
- Size-controlled

COMING SOON!

Reversible Cell Strainers (Catalog #27215/27250) are ideal for the filtration and isolation of cellular aggregates - including embryoid bodies (EBs) formed using AggreWell™ plates. Single cells are allowed to pass through the filter but EBs greater than 50 cells in size will be trapped. The Reversible Cell Strainer can then be turned over and placed stably onto a collection tube, and the aggregates can then be washed and collected.

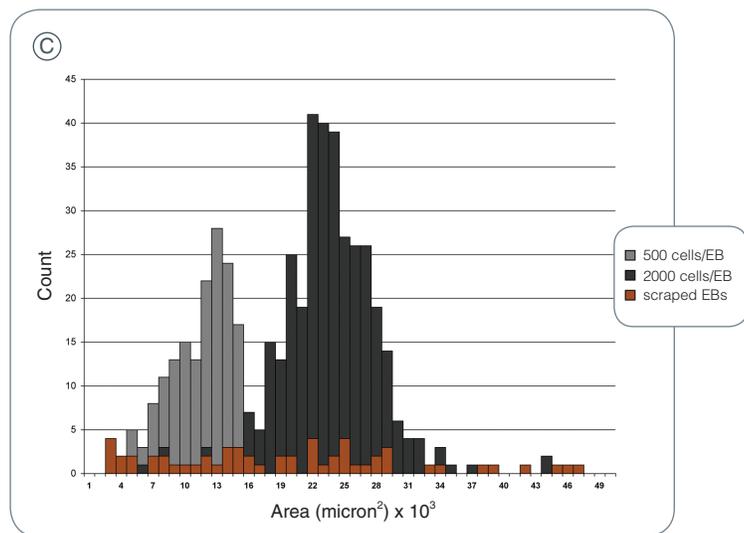
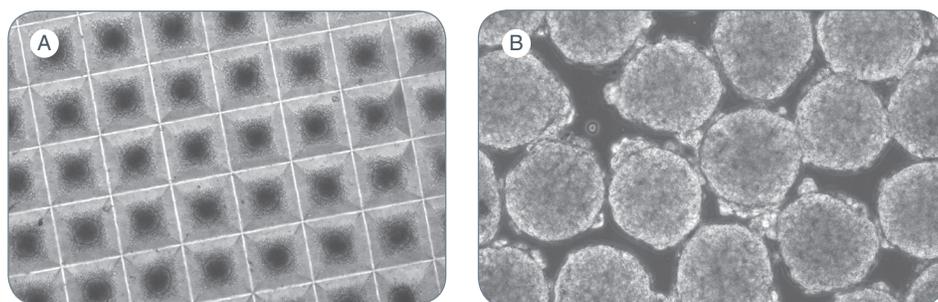


FIGURE 11:
(A) H9 hESCs were centrifuged into AggreWell™400 plates at a density of 2000 cells per microwell and cultured for 24 hours prior to EB harvest.
(B) The resulting EBs are uniform in size and shape.
(C) The size of EBs can be tightly controlled using AggreWell™400 plates, unlike scraping protocols which give a wider distribution.¹³

PRODUCT	DESCRIPTION	CATALOG #
40 µm Reversible Cell Strainer (15 mL)	Sterile, 40 µm nylon mesh filter, fits standard 14 mL round bottom tubes or 15 mL conical tubes	27215
40 µm Reversible Cell Strainer (50 mL)	Sterile, 40 µm nylon mesh filter, fits standard 50 mL conical tubes	27250

COMING
SOON!

SOLUTIONS FOR CELLS DIFFERENTIATED FROM HUMAN PLURIPOTENT STEM CELLS

Purify CD34⁺ Cells Differentiated From Human Pluripotent Stem Cells

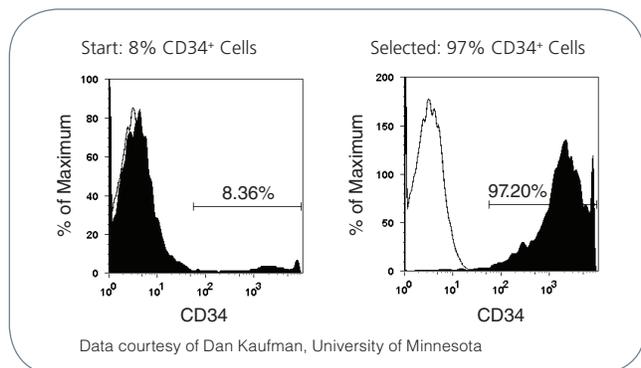


FIGURE 12: Typical FACS histogram results using EasySep[®] hESC-Derived CD34 Positive Selection Kit (Catalog #18167).

Generation of clinically-useful cell types derived from human pluripotent stem cells will rely on the optimization of relevant differentiation protocols. Due to issues with tumor formation of undifferentiated cells and non-specific differentiation, there is a need for methods to purify cell types of interest from a mixed cell population. EasySep[®] is a powerful immunomagnetic cell separation platform that combines the specificity of monoclonal antibodies with the simplicity of a column-free magnetic system for the gentle isolation of highly purified cells. The EasySep[®] hESC-derived CD34 Positive Selection Kit (Catalog #18167) provides an ideal way to purify CD34⁺ cells during hematopoietic differentiation protocols.

PRODUCT	DESCRIPTION	CATALOG #
EasySep [®] hESC-Derived CD34 Positive Selection Kit*	Optimized kit for the isolation of highly purified hESC-derived CD34 ⁺ cells Components: • EasySep [®] hESC-Derived CD34 Positive Selection Cocktail 1.0 mL • EasySep [®] Magnetic Nanoparticles 1.0 mL	18167
EasySep [®] Isolation of hESCs and hiPSCs using SSEA-4 Antibody Positive Selection Kit*	Optimized kit for the isolation of highly purified hESCs and hiPSCs using SSEA-4 Antibody	18087

NEW!

COMING SOON!

*Required equipment: EasySep[®] Magnet (Catalog #18000)

Additional Cell Separation Solutions for Cells Differentiated from Human Pluripotent Stem Cells:

EasySep[®] CUSTOM SELECTION KITS

Use any conjugated antibody with EasySep[®] PE (Catalog #18551), FITC (Catalog #18552), Biotin (Catalog #18553), or APC (Catalog #18451) Selection Kit to select or deplete your cells of interest.

RobsoSep[®] (CATALOG #20000)

Fully automated cell separator using EasySep[®] Technology for high throughput sample processing.

ADVANTAGES OF EasySep[®]

- **FAST AND EASY.** No columns or washes required.
- **HIGH PURITY AND RECOVERIES.** Purities of up to 97% with high recovery can be achieved.
- **FUNCTIONAL CELLS.** Gentle on cells allowing for the isolation of functional and viable cells.
- **FLOW CYTOMETRY COMPATIBLE.** Isolated cells are immediately available for use as EasySep[®] magnetic particles do not interfere with flow cytometry.

mFreSR® AND CryoStor™ CS10

DEFINED CRYOPRESERVATION MEDIA FOR HUMAN PLURIPOTENT STEM CELLS

mFreSR® Improves Thawing Efficiencies 5- to 10-Fold Over Other Reported Methods⁷⁻¹¹

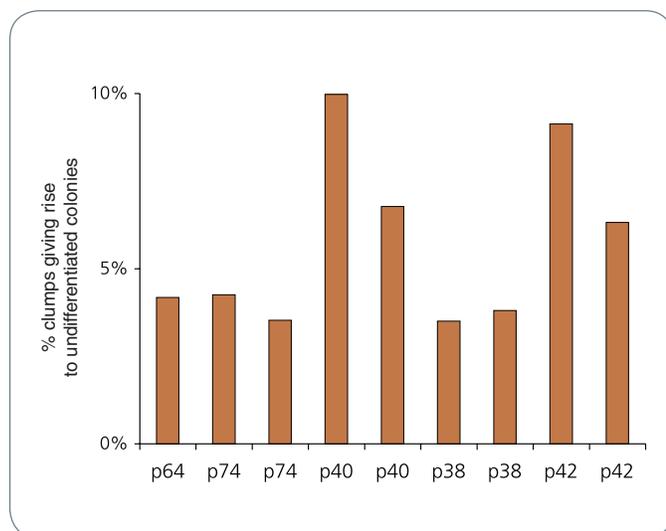


FIGURE 13: H9 hESCs were cryopreserved in mFreSR® at the indicated passage. Thawing efficiencies were analyzed by counting the number of surviving clumps after thawing.

Conventional cryopreservation methods for human pluripotent stem cells use fetal bovine serum. This introduces an undefined component into the culture media.

mFreSR® is a defined, cryopreservation medium designed specifically for use with hESCs and hiPSCs.

Together, mFreSR® and mTeSR®1 eliminate the two main sources of variability associated with hESC culture: serum and feeders.



PRODUCT	QUANTITY	CATALOG #
mFreSR®	10 x 5 mL	05854
	50 mL	05855

PRODUCT	QUANTITY	CATALOG #
CryoStor™ CS10	100 mL	07930

NEW!

CryoStor™ CS10 is an animal protein-free and defined cryopreservation medium for human ESCs and iPSCs.

SUPPORT REAGENTS

A variety of support products are available to accompany STEMCELL Technologies' array of specialized products for hESC and hiPSC research. Please visit www.stemcell.com for more details and a full list of tissue culture reagents and supplies.

Tissue Culture Media

PRODUCT	CATALOG #	UNIT SIZE
DMEM with 4500 mg/L D-glucose	36250	500 mL
DMEM with 1000 mg/L D-glucose	36253	500 mL
DMEM/F-12	36254	500 mL
Iscove's MDM (IMDM)	36150	500 mL

Balanced Salt Solutions

PRODUCT	CATALOG #	UNIT SIZE
D-PBS	37350	500 mL
D-PBS, 10X	37354	500 mL
HBSS, Ca ⁺⁺ & Mg ⁺⁺ free	37250	500 mL
HBSS, without Phenol Red	37150	500 mL

Enzymes

PRODUCT	CATALOG #	UNIT SIZE
ACCUTASE®	07920	100 mL
Collagenase	07902	5 mL
Collagenase Type IV	07909	100 mL
Dispase (1 mg/mL)	07923	100 mL
Dispase (5 mg/mL)	07913	100 mL
DNase I (1 mg/mL)	07900	1 mL
Trypsin-EDTA (0.05%)	07910	500 mL
Trypsin-EDTA (0.25%)	07901	500 mL
Trypsin in Citrate Saline	07400	100 mL

Antibiotics

PRODUCT	CATALOG #	UNIT SIZE
Penicillin G and Streptomycin, 100X	07500	100 mL
Neomycin (G418)	03812	250 mg
Hygromycin B	03813	100 mg

Miscellaneous Tissue Culture Reagents and Supplies

PRODUCT	CATALOG #	UNIT SIZE
3% Acetic Acid with Methylene Blue	07060	100 mL
Cell Strainers, 40 µm	27305	50/case
40 µm Reversible Cell Strainers (15 mL)	27215	COMING SOON!
40 µm Reversible Cell Strainers (50 mL)	27250	COMING SOON!
Collagen Solution (3 mg/mL)	04902	35 mL
Fibronectin (1 mg/mL)	07159	1 mL
Gelatin (0.1% in water)	07903	500 mL
Hypoxia Chamber	27310	1 chamber
Rat Serum	13551 13561	2 mL 5 x 2 mL
Sodium Pyruvate (100 mM)	07000	100 mL
Trypan Blue	07050	100 mL
Y-27632	07171	1 mg
	07172	5 mg

Tissue Culture Dishes

PRODUCT	CATALOG #	UNIT SIZE
35 mm diameter	27115	10/pack
	27116	500/case
60 mm diameter	27120	10/pack
	27121	400/case
100 mm diameter	27125	10/pack
	27127	240/case
245 mm x 245 mm	27140	4/pack
	27141	16/case
96-well plates	27135	1/pack
	27136	50/case

Recombinant Cytokines

CYTOKINE	CATALOG #	UNIT SIZE
Activin A	02514	5 µg
BAFF	02517	20 µg
Bone Morphogenetic Protein-2	02523	10 µg
Bone Morphogenetic Protein-4	02524	10 µg
Noggin	02525	20 µg
Wnt-3a	02527	10 µg
Basic Fibroblast Growth Factor (bFGF)	02634	25 µg
Transforming Growth Factor-β1	02647	2 µg
	02847	10 µg

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