

Products for Mouse Pluripotent Stem Cells

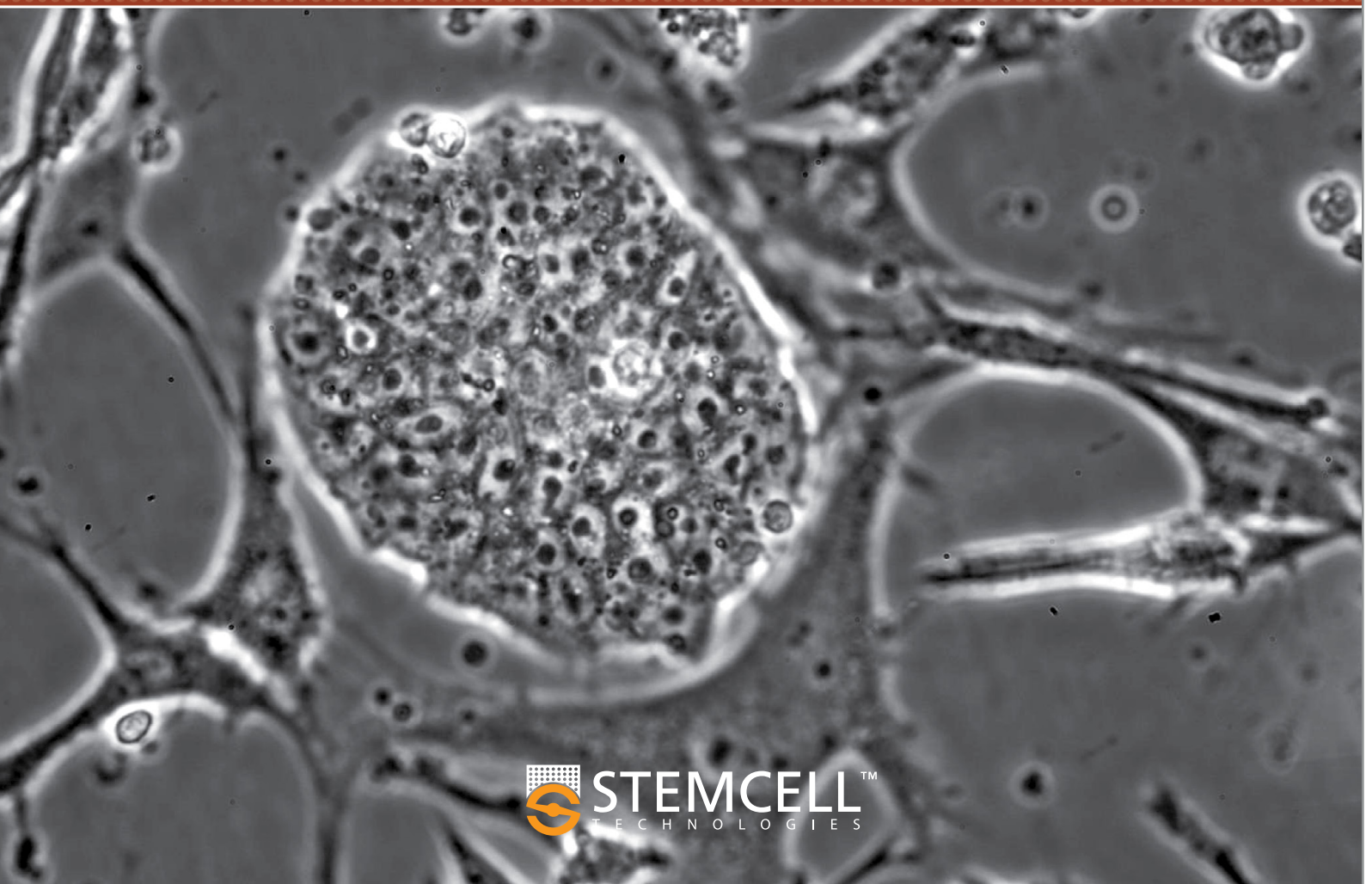


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Mouse Pluripotent Stem Cells

Products for Research

Introduction

Mouse embryonic stem (mES) cells are pluripotent cells derived from the inner cell mass of early blastocysts. They can be maintained in vitro for extended periods without loss of their capacity to contribute to all cell lineages when reimplanted back into a recipient blastocyst.¹⁻³ The pluripotency of mES cells, combined with their ease of genetic manipulation and selection, has revolutionized gene function in vivo through the generation of transgenic mice.⁴⁻⁹

In the fields of regenerative medicine and developmental biology, mES cells serve as a useful model system to investigate the genetic and epigenetic changes that take place during development.¹⁰⁻¹³

The discovery that it is possible to reprogram somatic cells into induced pluripotent stem (iPS) cells by overexpressing a small number of genes either through viral infection or by stimulation with small molecules, or both, has stimulated much interest in investigating the mechanisms responsible for this phenomena including various genetic and epigenetic changes that take place during this process.¹⁴⁻¹⁷ To date, these cells have been shown to be functionally indistinguishable from mES cells and are the subject of much research effort.

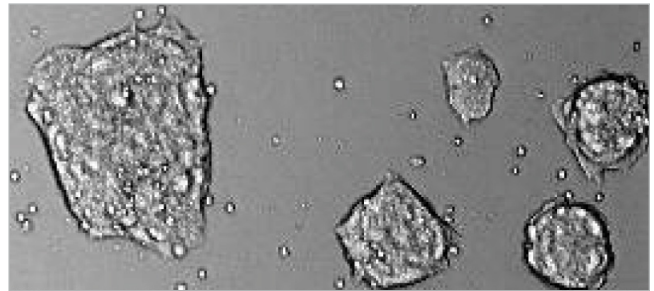
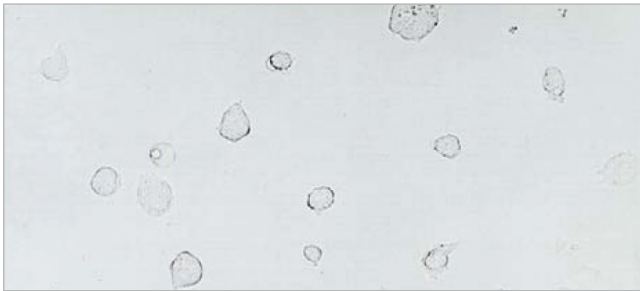


Morphology of Undifferentiated Mouse Embryonic Stem Cells

Undifferentiated mouse embryonic stem (mES) cells have a large nucleus, minimal cytoplasm, and one or more prominent dark nucleoli. It should be difficult to identify individual cells within the mES cell colony, as there are non-distinct cytoplasmic membranes between the cells. Colonies appear amorphous without a distinct or common shape. Signs of differentiation include the ability to distinguish individual cells within the mES cell colony by the defined cytoplasmic membrane for the cells. The colony may appear to spread and cells appear flattened. Cells may lift off of the dish.

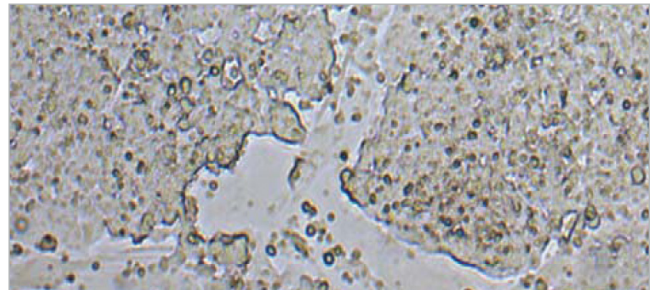
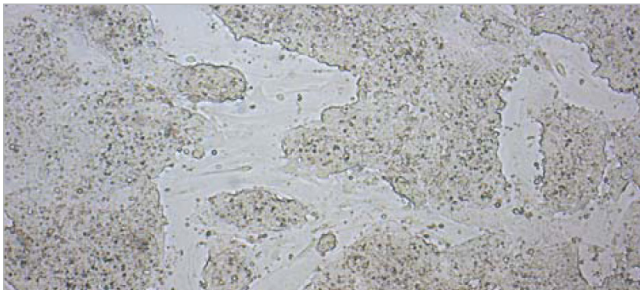
Undifferentiated mES Cells

Colonies are dense with distinct, tight borders, and individual cells are not visible. Colonies are not touching one another.



Differentiated mES Cells on MEFs

Colonies are merging, and have lost border integrity.



Differentiated mES Cells on Gelatin

Colonies are merging, and have lost border integrity.



Maintenance of

Undifferentiated mES & miPS cells



Mouse Recombinant LIF

Mouse Recombinant Leukemia Inhibitory Factor (LIF, Catalog #78056) is an interleukin 6 class cytokine that regulates a broad variety of developmental functions. It is recommended for the maintenance of mES cells.

COMPLETE mES & miPS CELL MAINTENANCE MEDIUM*			
Product	DES cellription	Quantity	Catalog #
Mouse Recombinant Leukemia Inhibitory Factor (mLIF)	Recommended for the maintenance of undifferentiated mES cells.	10 µg 50 µg 1000 µg	78056.1 78056 78056.2
DMEM with high glucose	Dulbecco's Modified Eagle's Medium with 4500 mg D-glucose/L. Contains sodium bicarbonate and sodium pyruvate.	500 mL	36250
MEM Non-Essential Amino Acids (10 mM)	Culture medium supplement for the maintenance of mES cells, supplied as 100X concentrated.	1000 µg	07600
L-Glutamine (200 mM)	Culture medium supplement for the maintenance of mES cells.	100 mL	07100

*Components essential for the preparation of complete mES & miPS cell maintenance medium are available as pre-screened ES-Cult™ products from STEMCELL Technologies. In addition to the products listed above, complete mES & miPS cell maintenance medium also requires monothioglycerol (e.g. Sigma #M6145) in a 1/100 working solution in DMEM (Catalog #36250) to a final concentration of 100 µM and fetal bovine serum pre-screened for the maintenance of mES and miPS cells.

Mouse Embryonic Fibroblasts (MEFs)

For Maintenance of mES & miPS Cells

CD-1 MEFs

Product	Passage	Day	Cell Number/Vial	Catalog #
CD-1 MEFs	2	E12.5	1 X 10 ⁶	00321
	2	E14.5	1 X 10 ⁶	00322

RECOMMENDED FOR:

The generation of feeder layers for the maintenance of undifferentiated mouse ES and iPS cells. Untreated MEFs must be mitotically inactivated by irradiation or mitomycin C treatment prior to forming feeder layers. MEFs are prepared from day E12.5 or E14.5 CD-1 mouse embryos, supplied at passage 2 and can be expanded up to passage 5. Each vial contains 1 x 10⁶ cells in DMEM with 50% fetal bovine serum and 10% dimethyl sulfoxide.

Selective Agents

Product	Quantity	Catalog #
G418	250 mg	03812
Hygromycin B	100 mg	03813
Puromycin	50 mg	73342
	500 mg	73344

Mouse Recombinant Leukemia Inhibitory Factor (LIF, Catalog #78056) is an interleukin 6 class cytokine that regulates a broad variety of developmental functions. It is recommended for the differentiation of mES cells.

Drug-Resistant MEFs

Product	Passage	Day	Cell Number/Vial	Catalog #
Neomycin-Resistant MEFs	1	E13.5	3 X 10 ⁶	00323
Hygromycin-Resistant MEFs	1	E13.5	3 X 10 ⁶	00324
Puromycin-Resistant MEFs	2	E13.5	3 X 10 ⁶	00325

RECOMMENDED FOR:

The generation of drug-resistant feeder layers for the culture and selection of transfected undifferentiated ES cells. MEFs and targeted ES cells can be co-cultured using neomycin/ G418, hygromycin or puromycin as a selective agent with the appropriate MEFs.

MEFs are isolated from day E13.5 drug-resistant mouse embryos, supplied at passage 1 or 2 and can be expanded up to passage 5. They must be mitotically inactivated by irradiation or mitomycin C treatment prior to forming feeder layers for ES cells. Each vial contains greater than 3 x 10⁶ cells in 95% fetal bovine serum and 5% dimethyl sulfoxide.

Additional Pre-Screened Products For Maintenance of Mouse ES & iPS Cells

PRODUCT	DES CELLRIPTION	QUANTITY	CATALOG #
Gelatin (0.1% in water)	Recommended for the coating of culture dishes or flasks.	500 mL	07903
Sodium Pyruvate (100 mM)	Only recommended to be added to mouse ES cell maintenance medium when ES-Cult™ DMEM high glucose medium (Catalog #36250) is NOT used.	100 mL	07000
Bone Morphogenetic Protein-4 (BMP-4)	Used together with mLIF to promote mouse ES cell self-renewal in serum-free medium. BMP-4 is also recommended for inducing hematopoietic activity and promoting hematopoietic precursor development during EB formation and in vitro mouse ES cell differentiation.	10 µg	02524
D-PBS without Ca ⁺⁺ and Mg ⁺⁺	Dulbecco's Phosphate Buffered Saline without Ca ⁺⁺ and Mg ⁺⁺ for rinsing mouse ES cell cultures prior to passage.	500 mL	37350
Trypsin-EDTA	0.25% Porcine Trypsin and 1 mM EDTA•4Na in Hanks' Balanced Salt Solution (Ca ⁺⁺ and Mg ⁺⁺ free). Recommended for detachment of adherent cells and dissociation of embryoid bodies.	500 mL	07901
Tissue Culture-Treated Dishes	Pre-tested for the ability to support the growth of anchorage-dependent cells. Available in 35 mm, 60 mm, and 100 mm formats.	500 Dishes	38046
Trypan Blue	0.4% in PBS. Recommended for viable cell counting.	100 mL	07050

Characterization

Products for mES & miPS Cells

Undifferentiated mES and miPS cells express high levels of OCT4 (OCT3) and SSEA-1 and begin to express SSEA-3 upon differentiation. Primary and appropriate secondary antibodies are available for detection of OCT4 (OCT 3), SSEA-1, SSEA-3 and SSEA-4.

PRIMARY ANTIBODIES			
Target Antigen	Clone	Isotype	Catalog #
OCT4 (OCT3)	40	Mouse IgG1	60059
SSEA-1 (CD15)	MC-480	Mouse IgM	60060
SSEA-3	MC-631	Rat IgM	60061
SSEA-4	MC-813-70	Mouse IgG3	60062

SECONDARY ANTIBODIES				
Target Antigen	Host Species	Format	For Use With	Catalog #
Mouse IgG	Goat	FITC	Anti-OCT4 (OCT3)	60138FI
Mouse IgM	Goat	FITC	Anti-SSEA-1 (CD15)	60139FI
Rat IgM	Goat	APC	Anti-Mouse SSEA-3 Antibody, Clone MC-631	60140AZ

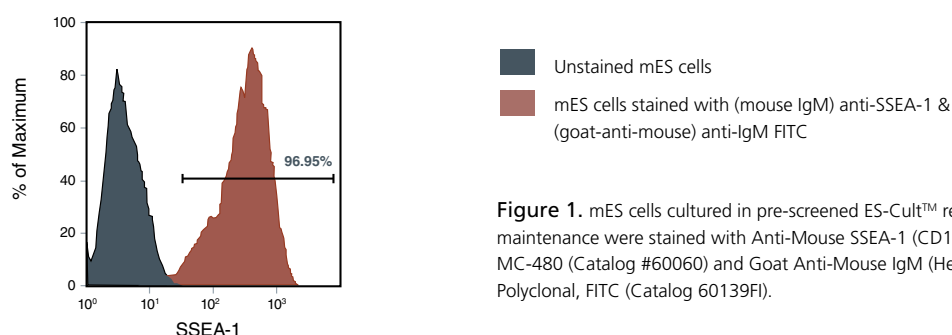


Figure 1. mES cells cultured in pre-screened ES-Cult™ reagents for maintenance were stained with Anti-Mouse SSEA-1 (CD15) Antibody, Clone MC-480 (Catalog #60060) and Goat Anti-Mouse IgM (Heavy Chain) Antibody, Polyclonal, FITC (Catalog 60139FI).

For a complete list of antibodies, including other conjugates, sizes and clones, please visit www.stemcell.com/antibodies.

Formation of Uniform Embryoid Bodies

Many pluripotent stem cell (PSC) differentiation protocols begin with the formation of 3-dimensional aggregates of cells called embryoid bodies (EBs). EB size affects subsequent differentiation trajectories, but conventional EB formation methods, such as scraping, can result in EBs which are heterogeneous in size and shape, leading to inefficient and uncontrolled differentiation.

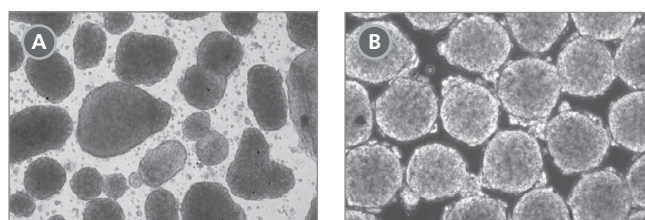
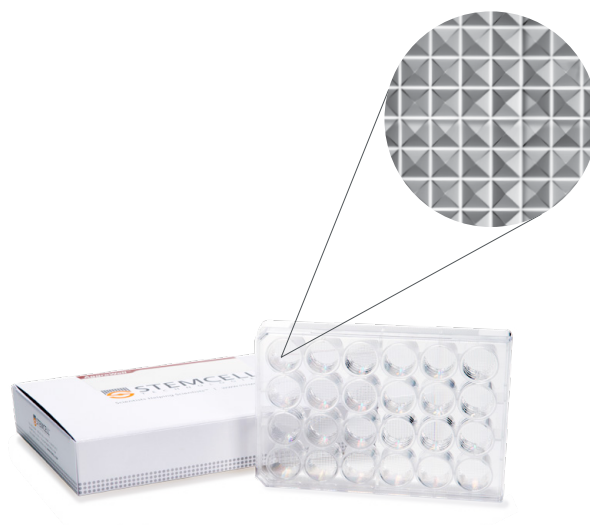


FIGURE 2. Generate Uniform Embryoid Bodies Using AggreWell™

(A) EBs formed using conventional methods are heterogeneous in size and shape resulting in inefficient differentiation. (B) EBs formed using AggreWell™ plates are uniform in size and consistently spherical in shape. Shown are EBs generated with 2,000 cells using AggreWell™400.



AggreWell™ plates are available in 2 sizes of microwells and multiple plate formats to fit your research needs.

For protocols which require defined EB size, we offer AggreWell™ plates, and for protocols which use semi-solid methylcellulose-based medium, we offer ES-Cult™ M3120.

1. AggreWell™

AggreWell™ plates contain microwells of defined size, providing an easy and standardized approach for the formation of size-controlled EBs. AggreWell™400 plates have microwells 400 μ m in size, and AggreWell™800 plates have microwells 800 μ m in size. Size of the EBs can be controlled by modifying the cell input density.

2. ES-Cult™ M3120 Base Methylcellulose Medium

Methods to support the differentiation of mES and miPS cells to either hematopoietic or endothelial cells involve the formation of EBs which have been derived from a single undifferentiated cell. ES-Cult™ M3120 (Catalog #03120) is a methylcellulose-based semi-solid medium which allows the progeny of individual cells to remain together. With the addition of appropriate cytokines, the resulting EBs will become either hematopoietic or endothelial cells. This method can also be used as a surrogate assay to show the number of undifferentiated cells in a culture.¹⁸

PRODUCT	MICROWELL SIZE	CELL RANGE	PLATE FORMAT	NUMBER OF SPHEROIDS	CATALOG #
AggreWell™400	400 μ m	50 - 3,000 cells per spheroid	24-well plate	~ 1,200 per well	34411/34415
			6-well plate	~ 7,000 per well	34421/34425
AggreWell™800	800 μ m	3,000 - 20,000 cells per spheroid	24-well plate	~ 300 per well	34811/34815
			6-well plate	~ 1,800 per well	34821/34825

Note: AggreWell™ Rinsing Solution (Catalog #07010) is required for use with AggreWell™ plates to ensure optimal performance.

Tissue Culture

Reagents & Supplies

MISCELLANEOUS TISSUE CULTURE REAGENTS AND SUPPLIES		
PRODUCT NAME	CATALOG #	QUANTITY
3% Acetic Acid with Methylene Blue	07060	100 mL
Collagen Solution	04902	35 mL
Fibronectin	07159	1 mL
Gelatin	07903	500 mL
Hypoxia Chamber	27310	1 Chamber
Rat Serum	13551 13561	2 mL 10 mL
Sodium Pyruvate	07000	100 mL
Trypan Blue	07050	100 mL

TISSUE CULTURE DISHES		
PRODUCT NAME	CATALOG #	QUANTITY
35 mm diameter	27115 27116	10/pack 500/case
60 mm diameter	27120 27121	10/pack 400/case
100 mm diameter	27125 27127	10/pack 240/case
245 mm x 245 mm	38039 100-0084	4/pack 16/case
96-well plates	27135 27136	1/pack 50/case

ANTIBIOTICS		
PRODUCT NAME	CATALOG #	QUANTITY
Neomycin (G418)	03812	250 mg
Hygromycin	03813	100 mg
Puromycin	73342	50 mg
	73344	500 mg

TISSUE CULTURE MEDIA		
PRODUCT NAME	CATALOG #	QUANTITY
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 NAME	CATALOG #	QUANTITY
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 NAME	CATALOG #	QUANTITY
ACCUTASE™	07920	100 mL
Collagenase	07902	5 mL
Collagenase Type IV	07909	100 mL
Dispase (1 mg/mL)	07923	100 mL
DNase I (1 mg/mL)	07900	1 mL
Trypsin-EDTA (0.25%)	07901	500 mL
Trypsin-EDTA (0.05%)	07910	500 mL

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