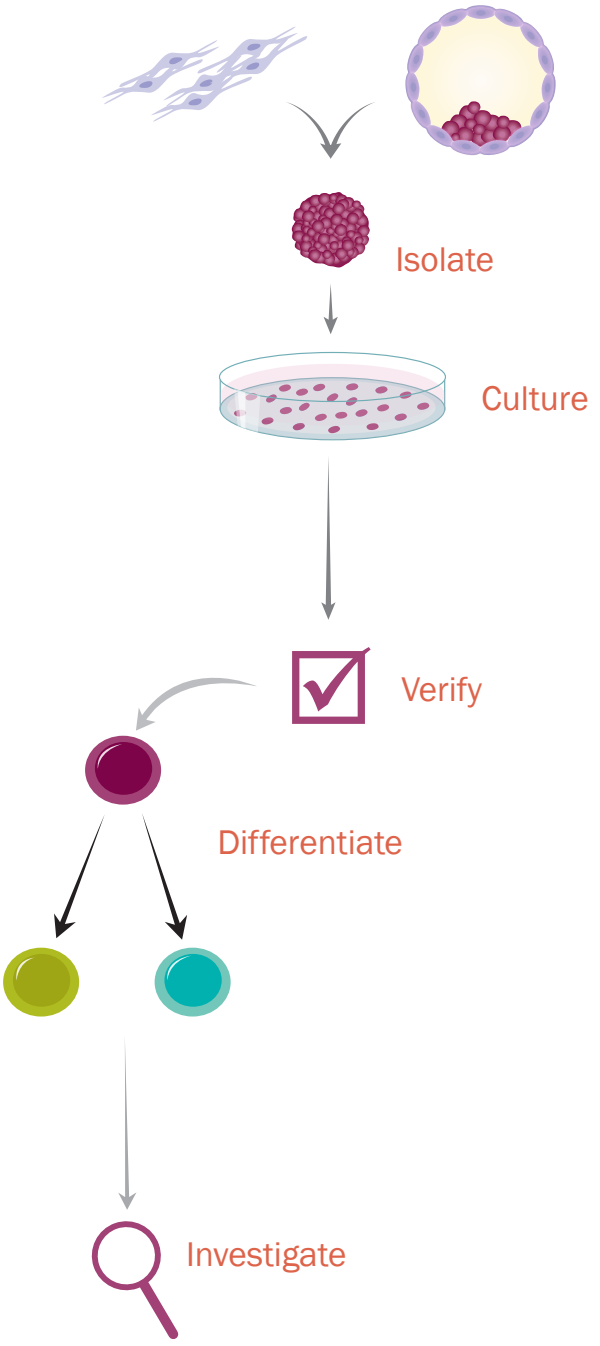
Embryonic and Induced Pluripotent Stem Cells



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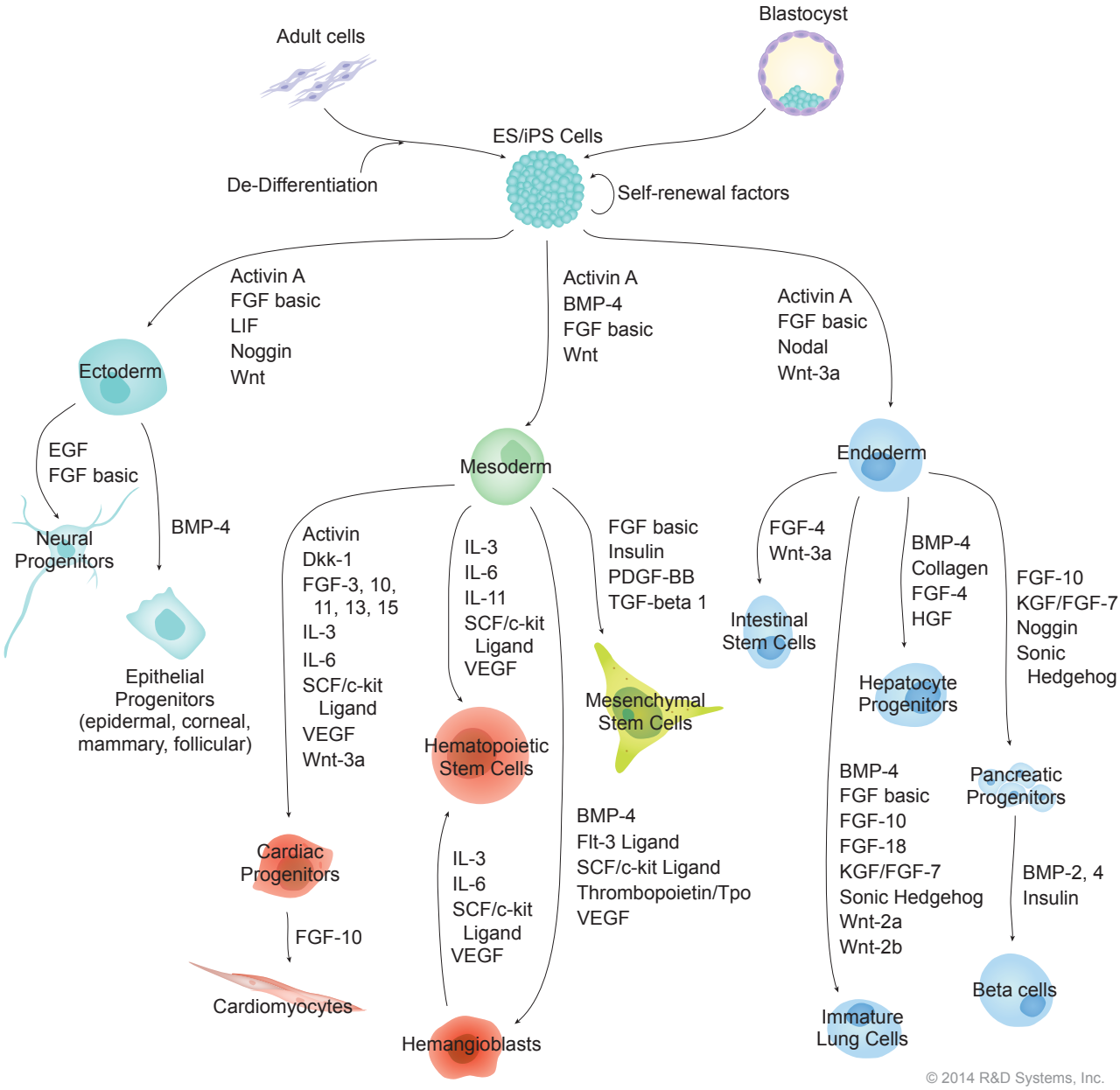
Embryonic and Induced Pluripotent Stem Cells

Embryonic stem (ES) cells and induced pluripotent stem (iPS) cells are self-renewing progenitors that have the capacity to differentiate into cells of the ectoderm, mesoderm, and endoderm. Naturally existing ES cells can be isolated from the inner cell mass of a blastocyst while iPS cells are generated by inducing expression of specific transcription factors in terminally differentiated somatic cells. ES/iPS cells hold enormous potential in basic and clinical research through their ability to differentiate into a wide variety of cell types, including neurons, pancreatic beta cells, cardiomyocytes, and progenitor cells of the liver, lung, and skin. ES/iPS self-renewal and differentiation are regulated by a precise temporal sequence of growth factor presentation, intracellular signaling, and transcription factor expression. As a result, ES/iPS cell research can require weeks of culture before hypotheses can be tested. R&D Systems and Tocris Bioscience present tools for the reliable isolation, differentiation, verification, and investigation of ES/iPS cells. These products will reduce experimental variability, improve data consistency, and prevent wasted effort and reagents.



Isolate and Culture

Pluripotent stem cells can be efficiently expanded in culture using specialized media and growth factors. It is important to begin experimentation with a verified, homogeneous, and pluripotent ES/iPS starter population to maintain confidence in cell differentiation and subsequent data interpretation. With this necessity in mind, R&D Systems offers the Human Pluripotent Stem Cell Starter Kit, a cost-effective option for the expansion and characterization of ES/iPS cells. We also offer high quality culture media, feeder-cells, culture matrix, and cryopreservation media for ES/iPS cell cultures.

Product	Catalog #
Human Pluripotent Stem Cell Starter Kit	SC029
Irradiated Mouse Embryonic Fibroblasts	PSC001
Mouse Embryonic Fibroblast Conditioned Media	AR005
StemXVivo™ Culture Matrix	CCM013
CryoDefend™-Stem Cells Media	CCM018

Human Pluripotent Stem Cell Starter Kit

Features

- **Complete** – an all-in-one kit for ES/iPS expansion and identification
- **Reliable** – uses feeder-free culturing conditions to reduce variability
- **Cost-effective** – includes premium quality media, growth factors, and antibodies
- Provides an ideal setup for researchers who are new to the stem cell field

Irradiated Mouse Embryonic Fibroblasts (iMEFs)

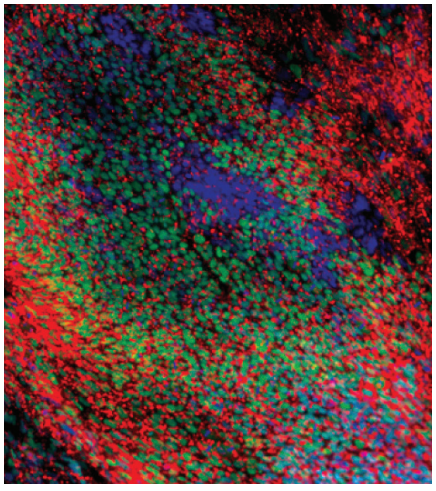
Features

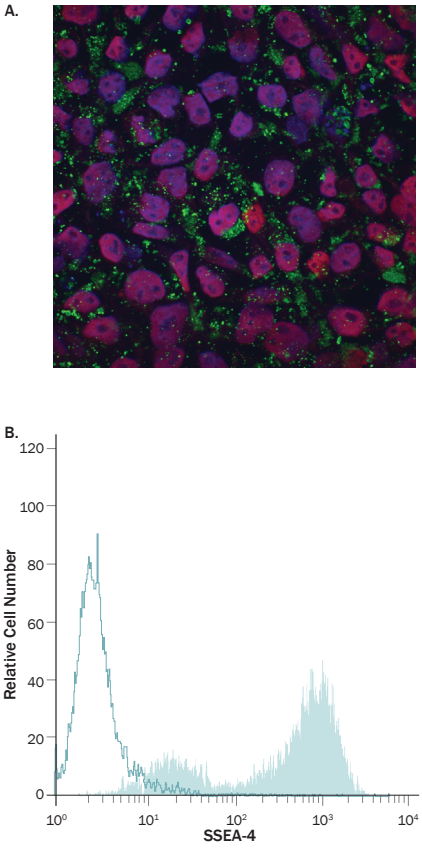
- **High quality** – ensures optimal growth, pluripotency, and viability of stem cell cultures
- **Consistent** – homogenous feeder layer reduces experimental variation
- **Ready to use** – free of microbial, mycoplasmal, and chemical contamination

Mouse Embryonic Fibroblasts (MEFs) Conditioned Media

Features

- **Established** – widely used culture system that eliminates the need for a MEF feeder layer
- **Consistent** – lot-to-lot consistency decreases experimental variability
- Generate complete media by adding FGF basic (Catalog # 233-FB)





Human iPS and ES Cells Cultured and Verified using Reagents in the Human Pluripotent Stem Cell Starter Kit. (**A**) The iPS cell line, iBJ6, was stained for pluripotent stem cell markers Nanog (red) and SSEA-4 (green) using primary antibodies supplied in the Human Pluripotent Stem Cell Starter Kit (Catalog # SC029). Primary antibodies were visualized with using NorthernLights™ (NL)557-conjugated Donkey Anti-Goat Secondary Antibody (Catalog # NL001) and NL493-conjugated Donkey Anti-Mouse Secondary Antibody (Catalog # NL009). The nuclei were counterstained with DAPI. (**B**) SSEA-4 expression was detected in BG01V human embryonic stem cells by flow cytometry. The cells were stained with PE-conjugated Goat Anti-Mouse Secondary Antibody (Catalog # F0102B; filled histogram) or Mouse IgG_3 Isotype Control Antibody (Catalog # MAB007; open histogram).

Human Pluripotent Stem Cells Cultured in MEF Conditioned Media Express Pluripotent Markers. Human ES cells were cultured in MEF Conditioned Media supplemented with Recombinant Human FGF basic (4 ng/mL; Catalog # 233-FB). SSEA-4 (red) was detected with a Mouse Anti-Human/Mouse SSEA-4 Monoclonal Antibody (Catalog # MAB1435) and NorthernLights (NL) 557-conjugated Donkey Anti-Mouse Secondary Antibody (Catalog # NL007). Oct-3/4 (green) was detected with a Goat Anti-Human Oct-3/4 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1759) and a NL493-conjugated Donkey Anti-Goat IgG Secondary Antibody (Catalog # NL003). The nuclei were counterstained with DAPI (blue).

StemXVivo Culture Matrix

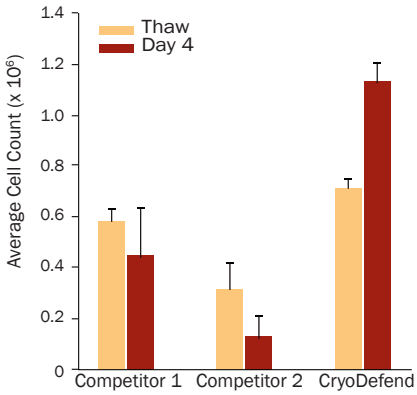
Features

- **Defined** – a proprietary mixture of R&D Systems premium quality recombinant human adhesion molecules
- **Quick** – straightforward coating procedure has plates ready for culture in 2–3 hours
- **Versatile** – validated in ES/iPS cells grown under high and low definition culture conditions

CryoDefend-Stem Cells Media

Features

- **Robust** – greater recovery of viable ES/iPS cells compared to conventional media
- **Consistent** – uses fully defined media to reduce experimental variability
- **Validated** – specifically designed and tested for use in stem cell cultures



Superior Human Pluripotent Stem Cell Recovery using CryoDefend-Stem Cells Media. BG01V human embryonic stem cells (1 x 10^6cells/cryovial) were frozen in CryoDefend-Stem Cells Media or cryopreservation media from two different competitors. Cells were thawed, immediately assessed for viability (orange bars), and resuspended for culture in Mouse Embryonic Fibroblast Conditioned Media (Catalog # AR005) containing Recombinant Human FGF basic (Catalog # 4114-TC). Cell viability was assessed again after four days in culture (red bars). The error bars indicate the standard deviation of triplicate samples.

Small Molecules for Reprogramming and Expansion

Small molecules can provide an additional level of control over iPS cell reprogramming as well as ES/iPS cell maintenance and expansion.

Reprogramming of Somatic Cells to iPSCs*		
Name	Description	Catalog #
CHIR 99021	Highly selective GSK-3 inhibitor	4423
3-Deazane-planocin A	Histone methyltransferase inhibitor; enhances Oct4 expression	4703
Forskolin	Adenylyl cyclase activator	1099
PD 0325901	Selective inhibitor of MEK1/2	4192
RepSox	Selective TGF-β RI inhibitor	3742
Thiazovivin	Improves the efficiency of fibroblast reprogramming	3845
TTNPB	Retinoic acid analog	0761

Maintenance of ESCs and iPSCs		
Name	Description	Catalog #
A 83-01	Selective inhibitor of TGF-β RI, ALK4 and ALK7	2939
BIO	Potent, selective GSK-3 inhibitor	3194
IWP 2	Inhibitor of Wnt processing	3533
PD 98059	MEK inhibitor	1213
Y-27632	Selective p160ROCK inhibitor; promotes survival of cryopreserved	1254

* Use of all 7 together found to reprogram somatic cells to iPS cells. Hou, P. *et al.* (2013) Science **341**:651.

Verify

Confidence in ES/iPS cell pluripotency prior to expansion and differentiation is essential for downstream experimentation and data interpretation. Beginning an experiment with suboptimal, unverified populations will put the investigator at risk for inconsistent results, thus wasting time and reagents. R&D Systems offers a selection of kits to verify ES/iPS cell pluripotency through either functional differentiation or cell-specific marker expression.

Product	Catalog #
Human Pluripotent Stem Cell Functional Identification Kit	SC027
Human Three Germ Layer 3-Color Immunocytochemistry Kit	SC022
GloLIVE™ Human Pluripotent Stem Cell Live Cell Imaging Kit	SC023
Human Pluripotent Stem Cell Marker Antibody Panel	SC008
Human Pluripotent Stem Cell Marker Antibody Panel Plus	SC009

Human Pluripotent Stem Cell Functional Identification Kit

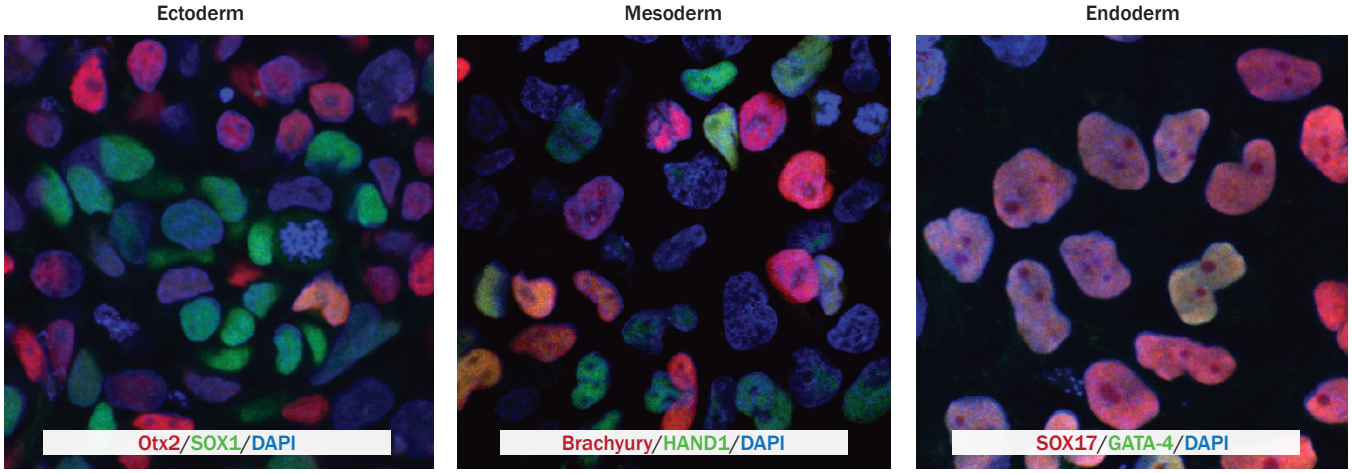
Features

- **Efficient** – differentiation and identification in 5 days
- **Reliable** – induces ES/iPS tri-lineage differentiation with kit-provided supplements
- **Complete** – contains germ layer-specific antibodies to confirm successful differentiation

Human Three Germ Layer 3-Color Immunocytochemistry Kit

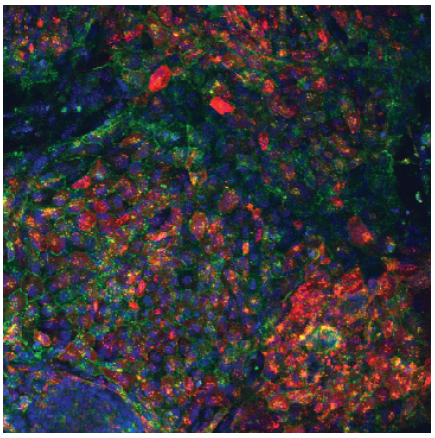
Features

- **Efficient** – includes fluorochrome-conjugated antibodies to detect ectoderm, mesoderm, and endoderm
- **Thorough** – contains 2 antibodies to confirm each tri-lineage cell-type
- **Fewer variables** – detects heterogeneity of starting cell populations



Functional Pluripotency of Human iPS Cells Confirmed with the Human Three Germ Layer 3-Color Immunocytochemistry Kit. Human iPS2 cells were differentiated into each of the three germ layers using the Human Pluripotent Stem Cell Functional Identification Kit (Catalog # SC027). Germ layer differentiation was verified using the six fluorochrome-conjugated antibodies provided in the Human Three Germ Layer 3-Color Immunocytochemistry Kit. Ectoderm differentiated cells were simultaneously stained with NorthernLights (NL)557-conjugated Otx2 (red) and NL493-conjugated SOX1 (green). Mesoderm differentiated cells were simultaneously stained with NL557-conjugated Brachyury (red) and NL637-conjugated HAND1 (green). Endoderm differentiated cells were simultaneously stained with NL637-conjugated SOX17 (red) and NL493-conjugated GATA-4 (green). All nuclei were counterstained with DAPI (blue). Nuclear expression of each marker was detected in their respective cell lineage.

GloLIVE Human Pluripotent Stem Cell Live Cell Imaging Kit



Features

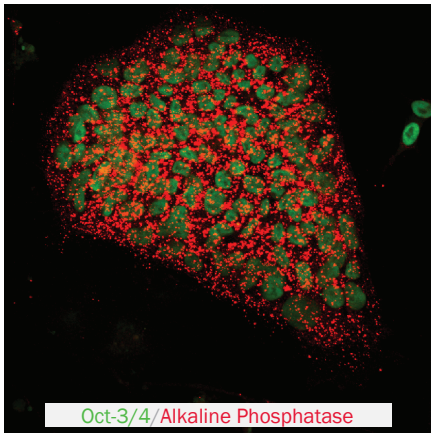
- **Quick** – verify pluripotency of live human stem cells in 30 minutes
- **Reliable** – pick pluripotent human stem cell colonies with increased confidence
- **Convenient** – no adverse effects on proliferation or differentiation following staining

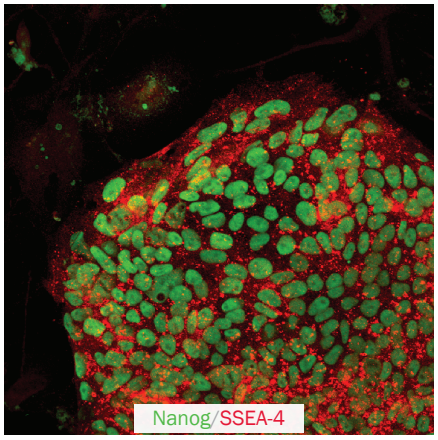
Verification of iPS Pluripotency using the GloLIVE Pluripotent Stem Cell Live Cell Imaging Kit. Human iPS2 cells were grown on Irradiated Mouse Embryonic Fibroblasts (Catalog # PSC001). NorthernLights (NL)493-conjugated SSEA-4 (green) and NL557-conjugated TRA-1-60(R) (red) antibodies from the GloLIVE Pluripotent Stem Cell Live Cell Imaging Kit were added to live cultures to label pluripotent stem cells. Cells were fixed and stained with Hoechst 33342 (blue) prior to imaging. Positive staining for SSEA-4 and TRA-1-60(R) combined with negative staining for SSEA-1 (not shown), indicates that cultures primarily contain undifferentiated human iPS cells.

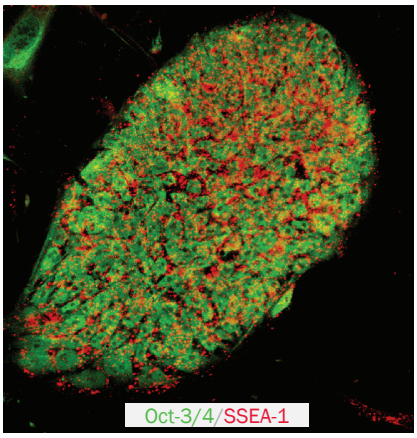
Human Pluripotent Stem Cell Marker Antibody Panel

Features

- **Cost-effective** – includes 5 high-quality antibodies specific for established ES/iPS cell markers
- **Validated** – manufactured and tested in-house for use with ES and iPS cells
- **Comprehensive** – increases confidence in pluripotency status through the use of multiple marker





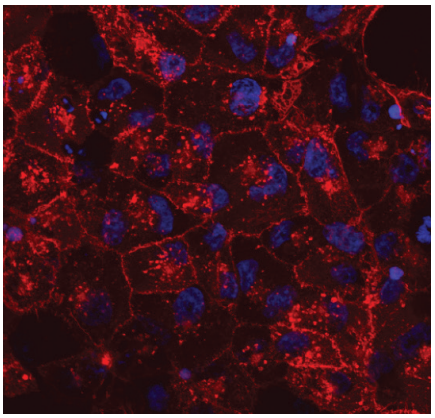


Expression of Pluripotency Markers in Human iPS Cells. Human iPS2 cells were grown on Irradiated Mouse Embryonic Fibroblasts (Catalog # PSC001) and labeled with antibodies provided in the Human Pluripotent Stem Cell Marker Antibody Panel (Catalog # SC008). Cells were visualized with specific combinations of NorthernLights (NL)486- and NL557-conjugated Secondary Antibodies as dictated by species requirements for each indicated primary antibody. This series of fluorescence images show iPS2 cells lack expression of SSEA-1 while retaining expression of positive pluripotency markers Oct-3/4, Alkaline Phosphatase, Nanog, and SSEA-4.

GloLIVE Antibodies

Azide-free, stem cell marker antibodies conjugated to NorthernLights (NL) fluorochromes that can be used for single-step, direct immunocytochemical staining of live, unfixed pluripotent stem cells.

Marker	Human Stem Cells	Mouse Stem Cells	Conjugated to NL493 (green)	Conjugated to NL557 (red)
SSEA-1	Negative Marker	Positive Marker	NLLC2155G	NLLC2155R
SSEA-4	Positive Marker	Negative Marker	NLLC1435G	NLLC1435R
TRA-1-60(R)	Positive Marker	Not applicable	NLLC4770G	NLLC4770R



Detection of SSEA-4 in Human ES cells. The pluripotent stem cell marker SSEA-4 was visualized in live BG01V human embryonic stem cells using the GloLIVE NL557-conjugated Mouse Anti-Human/Mouse SSEA-4 Monoclonal Antibody (Catalog # NLLC1435R; red). The cells were counterstained with Hoechst 33342 (blue).

Differentiate

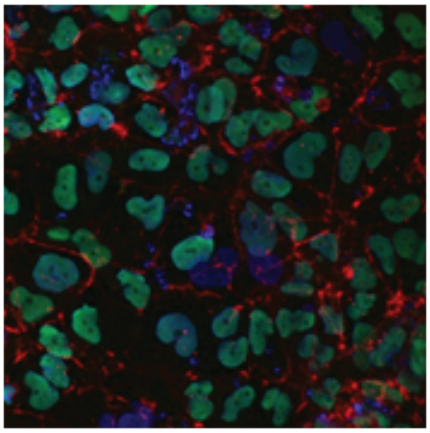
Efficient and consistent ES/iPS cell differentiation is essential for maximizing research productivity, increasing data reliability, and reducing the cost and labor associated with a sometimes lengthy differentiation process. These challenges are remedied by R&D Systems StemXVivo differentiation kits, which contain defined, premium quality factors to effectively drive differentiation of ES/iPS cells into ectoderm, mesoderm, and endoderm.

Human Pluripotent Stem Cell Differentiation Kits

Features

- **Robust** – yields highly enriched, differentiated cell populations in 3-4 days
- **Efficient** – reproducibly induces differentiation using optimized growth factors and supplements
- **Complete** – contains a marker antibody to verify differentiation status
- **Simple** – involves validated and straightforward procedures

Human Pluripotent Stem Cell Differentiation Kits	Catalog #
StemXVivo Ectoderm Kit	SC031
StemXVivo Mesoderm Kit	SC030
StemXVivo Endoderm Kit	SC019



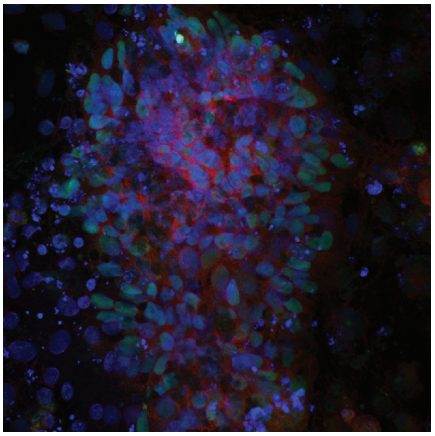
Differentiation of ES Cells into Endoderm Using the StemXVivo Endoderm Kit. BG01V human embryonic stem cells were differentiated into endoderm using media and supplements included in the kit. Cells were stained for the endoderm marker, SOX17 (green), and counterstained with Claudin-6 (red). SOX17 was visualized with the Goat Anti-Human SOX17 Antigen Affinity-purified Polyclonal Antibody included in the kit and the NorthernLights (NL)493-conjugated Donkey anti-Goat IgG Secondary Antibody (Catalog # NL003). Claudin-6 was visualized with the Mouse Anti-Human Claudin-6 Monoclonal Antibody (Catalog # MAB3656) and NL557-conjugated Donkey Anti-Mouse IgG Secondary Antibody (Catalog # NL007). The nuclei were labeled with DAPI (blue).

Small Molecules for Differentiation

Features

- Gain temporal control of differentiation pathways
- Modulate cell fate by targeting specific signaling pathways
- Minimize the use of animal-derived factors

ES and iPS Differentiation		
Name	Description	Catalog #
DMH-1	Promotes neurogenesis	4126
TWS 119	Promotes neurogenesis	3835
KY 02111	Promotes cardiomyogenesis	4731
1-EBIO	Promotes cardiomyogenesis	1041
IDE 1	Induces definitive endoderm formation	4015
Retinoic acid	Promotes ESC differentiation	0695
Exendin-4	Potentiates insulin secretion	1933
Cyclopamine	Induces ES differentiation into endocrine cells	1623



Small Molecules Promote iPS Cell Differentiation into Cells of Pancreatic Lineage. Cells from the iPS cell line, iBJ7, were differentiated into pancreatic cells using a combination of growth factors as well as a series of small molecules, including Retinoic acid (Catalog # 0695), Cyclopamine (Catalog # 1623), and Exendin-4 (Catalog # 1933). iBJ7 cells were allowed to differentiate for 20 days and then stained with Mouse Anti-Human Insulin C-Peptide Monoclonal Antibody (red; Catalog # MAB14171) and Goat Anti-Human SOX17 Polyclonal Antibody (green; Catalog # AF1924). Primary antibodies were visualized using Donkey Anti-Mouse NL557-conjugated (Catalog # NL007) and Donkey Anti-Goat NL493-conjugated (Catalog # NL003) secondary antibodies, respectively. The nuclei were labeled with DAPI (blue).

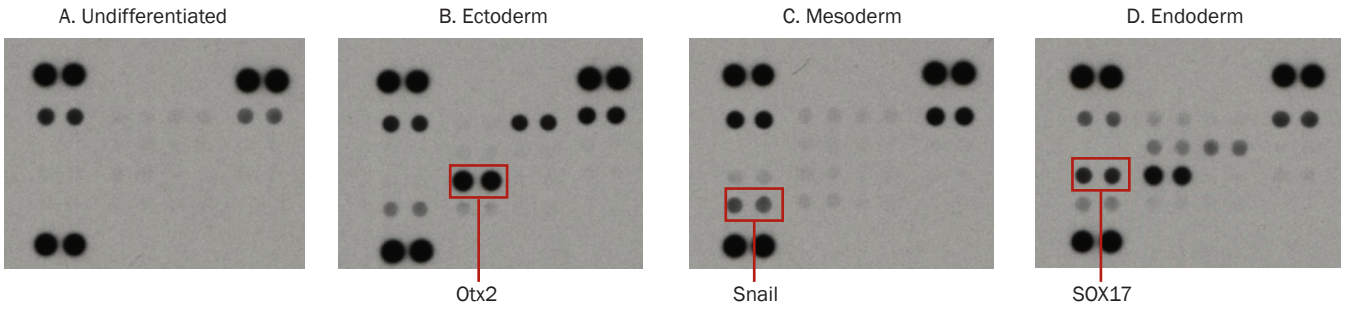
Investigate

After carefully validating ES/iPS pluripotency, expanding population size, and driving differentiation R&D Systems offers tools to investigate the function of terminally differentiated stem cells. Utilize our Proteome Profiler™ Array Kits to perform expedited protein analysis on your cells or explore our vast selection of high quality antibodies validated for various scientific applications, including immunocytochemistry and flow cytometry.

Proteome Profiler™ Human Pluripotent Stem Cell Array Kit

Features

- **Rapid** – analyze the expression level of dozens of proteins simultaneously
- **Economical** – contains 4 membranes – each protein is spotted in duplicate
- **Convenient** – small sample size requirements



Proteome Profiler Uses Small Lysate Volume to Analyze Markers of Human Pluripotent Stem Cell Differentiation. BG01V human embryonic stem cells were differentiated into ectoderm, mesoderm, and endoderm using the differentiation supplements included in the Human Pluripotent Stem Cell Functional Identification Kit (Catalog # SC027). Following differentiation in a 24-well plate, a single well of confluent cells was lysed in 100 µL of lysis buffer and analyzed using the Proteome Profiler Human Pluripotent Stem Cell Array Kit (Catalog # ARY010). The differentiation supplements upregulated expression of markers for ectoderm (**B**; Otx2), mesoderm (**C**; Snail), and endoderm (**D**; SOX17) compared to undifferentiated cells (**A**).

Antibodies

Whether you are characterizing ES/iPS populations or investigating protein expression in your terminally differentiated cells, R&D Systems vast catalog of over 13,000 primary antibodies and more than 150 isotype control/secondary antibodies will generate robust and trustworthy data.

Features

- **Diverse** – wide selection available for pluripotent stem cells and their differentiated progeny
- **Reliable** – rigorous quality testing ensures lot-to-lot consistency and outstanding performance
- **Specific** – tested for cross-reactivity with related molecules by direct ELISA

Learn more | RnDSystems/ESiPS_Markers

BG01V human embryonic stem cells are licensed from ViaCyte, Inc.

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