STEMdiff™ Trilineage Differentiation Kit

Functional assay kit to assess pluripotency by directed differentiation of human ES and iPS cells to all three germ layers

Catalog #05230 1 Kit

STEMCELL™ TECHNOLOGIES
Scientists Helping Scientists™ | WWW.STEMCELL.COM
TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713
INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM
FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

Product Description

The STEMdiff™ Trilineage Differentiation Kit provides a simple culture assay to functionally validate the ability of new or established human embryonic stem (ES) and induced pluripotent stem (iPS) cell lines to differentiate to the three germ layers: ectoderm, mesoderm, and endoderm. This kit includes specialized, complete media and monolayer-based protocols to perform parallel in vitro directed differentiation experiments for each germ layer, clearly and reproducibly establishing trilineage differentiation potential within one week.

STEMdiff™ Trilineage Differentiation Kit is intended to be an endpoint assay and is not optimized for the generation of cells for downstream differentiation or other applications. STEMdiff™ Trilineage Differentiation Kit has been optimized to assess cells maintained in mTeSR™1.

Product Information

The following components are sold as a complete kit (Catalog #05230) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
STEMdiff™ Trilineage Ectoderm Medium	05231	175 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
STEMdiff™ Trilineage Mesoderm Medium	05232	100 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
STEMdiff™ Trilineage Endoderm Medium	05233	100 mL	Store at -20°C.	Stable until expiry date (EXP) on label.

Materials Required But Not Included

PRODUCT NAME	CATALOG #
mTeSR™1	85850
DMEM/F-12 with 15 mM HEPES	36254
D-PBS (Without Ca++ and Mg++)	37350
Gentle Cell Dissociation Reagent	100-0485
Corning® Matrigel® hESC-Qualified Matrix	Corning 354277
Y-27632	72302
Trypan Blue	07050

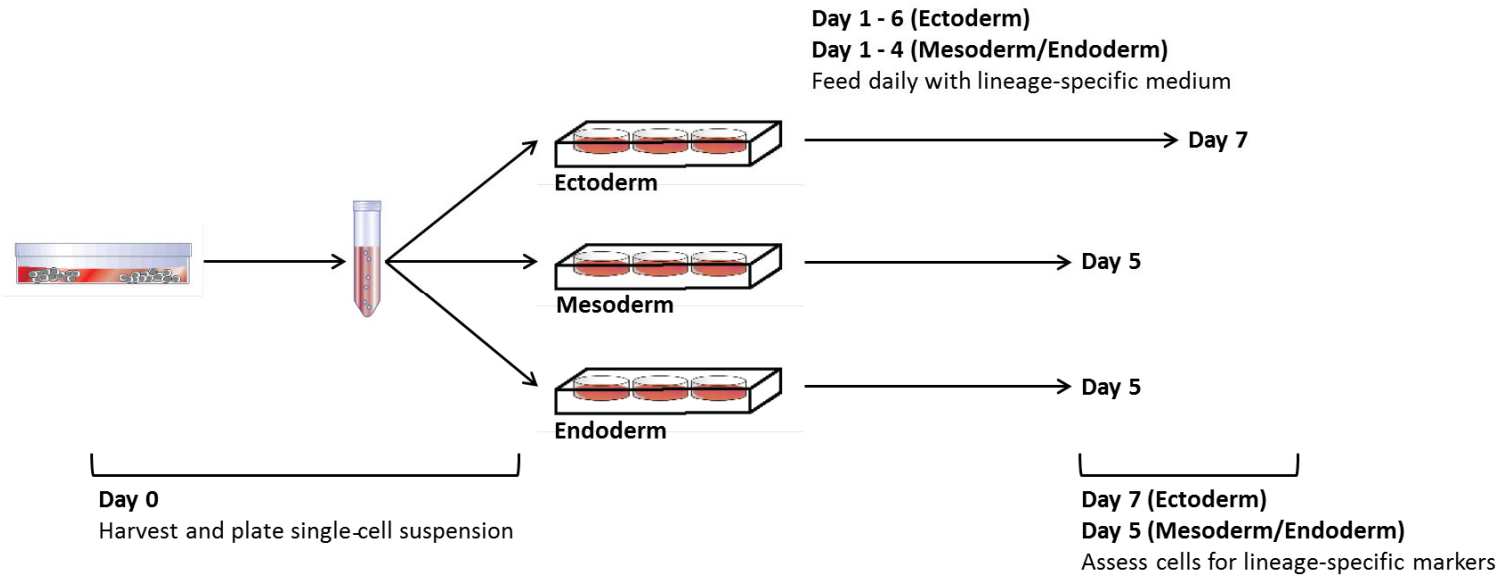
Preparation of Media

STEMdiff™ Trilineage Ectoderm, Mesoderm, and Endoderm Media

Thaw at room temperature (15 - 25°C) or overnight at 2 - 8°C. Mix thoroughly.

NOTE: Do not filter medium. Once thawed, use immediately or store at 2 - 8°C for up to 2 weeks for Ectoderm and Mesoderm Medium or 1 week for Endoderm Medium. Alternatively, aliquot into polypropylene or PET-E tubes or bottles and store at -20°C. Do not exceed the expiry date as indicated on the label. After thawing the aliquots, use immediately or store at 2 - 8°C for up to 2 weeks for Ectoderm and Mesoderm Medium or 1 week for Endoderm Medium. Do not re-aliquot into additional tubes or bottles or re-freeze.

Protocol Diagram



Directions for Use

Please read the entire protocol before proceeding.

The following protocol is for generating cells of ectoderm, mesoderm, and endoderm lineages from human ES or iPS cells previously cultured in mTeSR™1. Human ES/iPS cells should be used for the differentiation assay when they are ready for passaging. The majority of colonies should be large, compact, and exhibit multi-layering at the center.

NOTE: For complete instructions on coating plates with Corning® Matrigel® and maintaining high-quality human ES and iPS cells for use in differentiation, refer to the Technical Manual: Maintenance of Human Pluripotent Stem Cells in mTeSR™1, available at www.stemcell.com or contact us to request a copy.

A. PLATING HUMAN ES/iPS CELLS FOR TRILINEAGE DIFFERENTIATION

The following instructions are for passaging human ES/iPS cells from wells of a 6-well plate to set up the Trilineage Differentiation assay in 24-well plates. If using alternative cultureware, adjust volumes accordingly.

Coat cultureware with Corning® Matrigel® hESC-Qualified Matrix and bring to room temperature (15 - 25°C) for at least 30 minutes prior to use.

1. On day 0, warm to room temperature sufficient volumes of mTeSR™1, DMEM/F-12, and Gentle Cell Dissociation Reagent for passaging.
2. Prepare Single-Cell Plating Medium by adding Y-27632 to mTeSR™1 to reach a final concentration of 10 µM.
3. Use a microscope to visually identify regions of differentiation in the wells to be passaged. Mark these using a felt tip or lens marker on the bottom of the plate. Remove regions of differentiation by scraping with a pipette tip or by aspiration.
4. Wash each well to be passaged with 1 mL of D-PBS (Without Ca++ and Mg++).
5. Aspirate the wash medium and add 1 mL/well of Gentle Cell Dissociation Reagent.
6. Incubate at 37°C for 8 - 10 minutes.
7. In each well, dislodge cells by pipetting up and down 1 - 3 times using a pipette with a 1 mL tip. Ensure all remaining cell aggregates are broken up into single cells.
8. Immediately transfer cells to a tube containing 1 mL of DMEM/F-12 per well harvested. Wash each well once with 1 mL of DMEM/F-12 to collect any remaining cells and transfer to the tube. Centrifuge the tube at 300 x *g* for 5 minutes.
9. Remove supernatant. Resuspend cells in 1 mL Single-Cell Plating Medium (prepared in step 2).
10. Count viable cells using Trypan Blue and a hemocytometer.
11. Aspirate Matrigel® from coated 24-well plates. Add 0.5 mL of Single-Cell Plating Medium per well. Recommended volumes for plating in various types of cultureware are indicated in Table 1.

Table 1. Recommended Volumes of STEMdiff™ Trilineage Medium (Ectoderm, Mesoderm, or Endoderm) for Various Cultureware

CULTUREWARE	VOLUME OF SINGLE-CELL PLATING MEDIUM (section A)	FEED VOLUME OF STEMdiff™ TRILINEAGE MEDIUM (section B)
24-well plate	0.5 mL/well	1 mL/well
12-well plate	1 mL/well	1.5 mL/well
6-well plate	2 mL/well	3 mL/well

12. Add the appropriate number of cells to the medium-containing wells. The required densities for each lineage are indicated in Table 2.

Table 2. Plating Densities for Ectoderm, Mesoderm, and Endoderm Lineages

LINEAGE	CELL DENSITY (cells/cm^2)	TOTAL NUMBER OF CELLS PER WELL		
		24-well plate	12-well plate	6-well plate
Ectoderm	200,000	400,000	800,000	2,000,000
Mesoderm	50,000	100,000	200,000	500,000
Endoderm	200,000	400,000	800,000	2,000,000

13. Place the plate in a 37°C incubator. Move the plate in several quick, short, back-and-forth and side-to-side motions to evenly distribute the cells. Do not disturb the plate for 24 hours.
14. Continue to section B for differentiation.

B. DIFFERENTIATING MONOLAYER CULTURES TO THREE GERM LINEAGES

1. On **day 1**, warm the three types of STEMdiff™ Trilineage media (see Preparation of Media) to room temperature (15 - 25°C).
2. Aspirate media from cell cultures.
3. Add the appropriate STEMdiff™ Trilineage medium to each well. Recommended feed volumes for various types of cultureware are shown in Table 1.
4. Incubate at 37°C for 24 hours.
5. Repeat steps 1 - 4 until **day 5** (mesoderm and endoderm lineages) or **day 7** (ectoderm lineage).
6. Proceed to section C for assessing differentiation.

C. ASSESSING DIFFERENTIATION TO THREE GERM LINEAGES

Cells should be harvested and/or fixed for analysis of lineage-specific markers on **day 5** for mesoderm and endoderm lineages and **day 7** for the ectoderm lineage. The analysis method is determined by the user and may include flow cytometry, immunocytochemistry, or transcriptome analysis. If assessing for differentiation by flow cytometry or immunocytochemistry, label with combinations of fluorochrome-conjugated antibodies specific for early differentiation for each germ layer, for example:

- Ectoderm: PAX6 combined with Nestin (e.g. Anti-Human Nestin Antibody, Clone 10C2, Catalog #60091)
- Mesoderm: Brachyury (T) combined with either NCAM or CXCR4 (e.g. Anti-Human CD56 [NCAM] Antibody, Clone HCD56, Catalog #60021; or Anti-Human CD184 [CXCR4] Antibody, Clone 12G5, Catalog #60089)
- Endoderm: SOX17 combined with either CXCR4 or FOXA2

Related Products

For related products, including specialized media, matrices, antibodies, cytokines, and small molecules, visit www.stemcell.com/hPSCworkflow or contact us at techsupport@stemcell.com.