STEMdiff™ Lung Progenitor Kit

Serum-free medium for the differentiation of human ESCs and iPSCs to lung progenitor cells

Catalog #100-0230 1 Kit

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Product Description

STEMdiff™ Lung Progenitor Kit is a serum-free medium system for efficient and reproducible generation of lung progenitor cells from human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) through three stages of differentiation: 1) definitive endoderm, 2) anterior foregut endoderm, and 3) lung progenitor cells. Differentiated cells will express NKX2.1, a key marker of lung progenitor cells. The resulting cells can be further matured towards proximal or distal airway cells using published protocols for the study of lung diseases and lung development.

STEMdiff™ Lung Progenitor Kit has been optimized for differentiation of cells maintained in mTeSR™1 (Catalog #85850) or mTeSR™ Plus (Catalog #100-0276).

Product Information

The following components are sold as a complete kit (Catalog #100-0230) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
STEMdiff™ Definitive Endoderm Supplement CJ (100X)	05113	1.1 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
STEMdiff™ Definitive Endoderm Supplement MR (100X)	05112	0.35 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
STEMdiff™ Endoderm Basal Medium (Lung)*	100-0224	100 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
STEMdiff™ Lung Basal Medium	100-0196	180 mL	Store at 2 - 8°C.	Stable until expiry date (EXP) on label.
STEMdiff™ Lung Progenitor Supplement 1 (100X)**	100-0231	0.55 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
STEMdiff™ Lung Progenitor Supplement 2 (100X)	100-0232	1.45 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
STEMdiff™ Lung Supplement (10X)*	100-0197	20 mL	Store at -20°C.	Stable until expiry date (EXP) on label.

*This component contains material derived from human plasma. Donors have been tested and found negative for HIV-1 and -2, hepatitis B, and hepatitis C prior to donation. However, this product should be considered potentially infectious and treated in accordance with universal handling precautions.
**Please refer to the Safety Data Sheet (SDS) for hazard information. This product contains components dissolved in dimethyl sulfoxide (DMSO). DMSO is a strong solvent and skin penetrant, and can transport many substances through the skin. DMSO can also penetrate some protective glove materials including latex and silicone. Extra caution should be utilized when handling this product.

Materials Required but Not Included

PRODUCT NAME	CATALOG #
15 mL or 50 mL conical tubes	e.g. 38009 or 38010
Corning® Matrigel® hESC-Qualified Matrix	Corning 354277
Costar® 24-Well Flat-Bottom Plate, Tissue Culture-Treated	38017
D-PBS (Without Ca++ and Mg++)	37350
Gentle Cell Dissociation Reagent	100-0485
mTeSR™1	85850
mTeSR™ Plus	100-0276
Y-27632 (Dihydrochloride)	72302

Directions for Use

Please read the entire protocol before proceeding.

NOTE: For complete instructions on coating plates with Corning® Matrigel® and maintaining high-quality hESCs and hiPSCs for use in differentiation, refer to the Technical Manual: Maintenance of Human Pluripotent Stem Cells in mTeSR™1 (Document #10000005505) or mTeSR™ Plus (Document #10000007758), available at www.stemcell.com, or contact us to request a copy. Coated plates should be prepared in advance and brought to room temperature (15 - 25°C) for at least 30 minutes prior to use.

Use sterile technique when performing the following protocols:

I. Passaging hESCs/hiPSCs

II. Differentiation of hESCs/hiPSCs to Lung Progenitor Cells

- A. Protocol Diagram
- B. Preparation of Media
- C. Differentiation Protocol

I. PASSAGING HUMAN hESCs/hiPSCs

The following protocol is for clump passaging hESCs or hiPSCs cultured in mTeSR™1 or mTeSR™ Plus from one well of a 6-well plate to one well of a 24-well plate. If using other cultureware, adjust volumes accordingly. It is critical that the cells are of high quality (< 5% differentiation).

NOTE: hESCs and hiPSCs are ready for passaging when cultures are approximately 70% confluent.

1. Coat a tissue culture-treated 24-well plate with Corning® Matrigel®.
2. Aliquot a sufficient volume of mTeSR™1 or mTeSR™ Plus and warm to room temperature (15 - 25°C).
3. Use a microscope (4X magnification) to visually identify regions of differentiation in the hESC/hiPSC culture by marking them using a felt tip or lens marker on the bottom of the plate.
4. Remove these regions of differentiation by scraping with a pipette tip or by aspiration. Avoid having the culture plate out of the incubator for more than 15 minutes at a time.

   NOTE: Removal of differentiated cells will result in better differentiation efficiency.
5. Aspirate medium from the well and add 1 mL of Gentle Cell Dissociation Reagent (GCDR). Incubate at room temperature for 4 - 7 minutes.

   NOTE: Incubation times may vary when using different cell lines or other non-enzymatic cell passaging reagents (e.g. ReLeSR™, Catalog #100-0484); dissociation should be monitored under the microscope until the optimal time is determined.
6. Aspirate GCDR and add 1 mL of mTeSR™1 or mTeSR™ Plus. Gently detach the colonies by scraping with a cell scraper/lifter.

   NOTE: Take care to minimize the breakup of colonies.
7. Transfer the detached cell aggregates to a 15 mL or 50 mL conical tube.

   OPTIONAL: Rinse the well with an additional 1 mL of mTeSR™1 or mTeSR™ Plus to collect remaining cell colonies.

   NOTE: Centrifugation of cell aggregates is not required.
8. Carefully pipette the cell clump mixture up and down in a slow motion to break up the colonies using either a 1 mL pipettor or a 2 mL serological pipette. A uniform suspension of cell clumps approximately 50 - 200 µm in size is optimal. Avoid creating a single-cell suspension.
9. Gently agitate the cell suspension to ensure cell aggregates are evenly distributed. Transfer 5 µL of clump suspension into one well of a flat-bottom 96-well plate containing 50 µL D-PBS (Without Ca++ and Mg++). Count the total number of clumps (50 - 200 µm in diameter) in the well.

   NOTE: If most cell aggregates are > 200 µm in diameter, repeat steps 8 - 9.
10. Calculate the volume (in µL) of clump suspension required to seed 5000 clumps as follows:

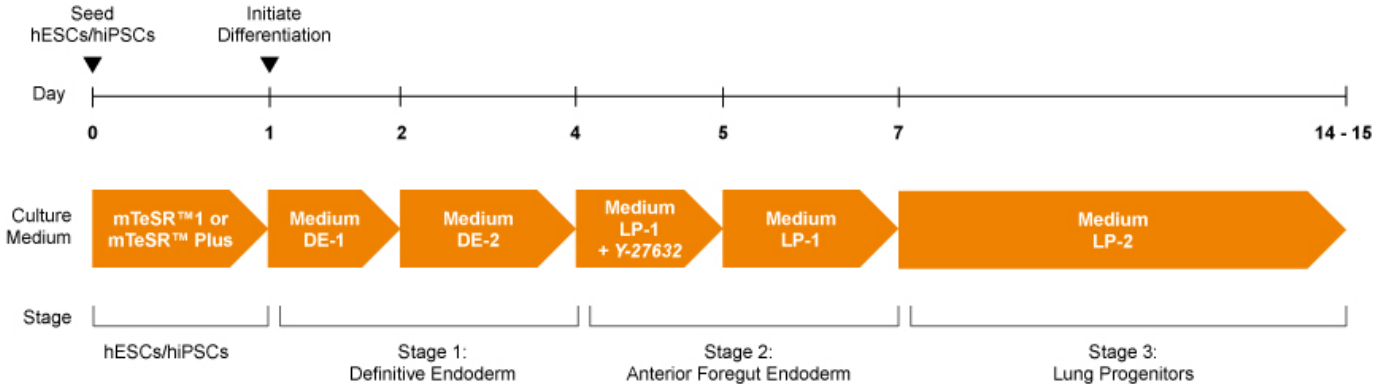
$$Volume\ of\ clump\ suspension\ (\mu L) = 5000\ cell\ clumps\ \div \frac{Number\ of\ clumps\ in\ 5\ \mu L}{5\ \mu L}$$

11. Add 0.5 mL mTeSR™1 or mTeSR™ Plus per well of the Matrigel®-coated 24-well plate prepared in step 1. Add the appropriate volume of clump suspension for 5000 clumps (calculated in step 10) to each well containing medium. Incubate at 37°C with 5% CO_2 and 95% humidity. Ensure cells are evenly distributed within each well by rocking the plate in a back-and-forth and side-to-side motion a few times as the plate is being placed in the incubator. Do not disturb the plate for 24 hours.

    NOTE: An initial experiment is recommended to determine the optimal clump-seeding density for the cell line being used. Seed a range of clump densities (e.g. 1000, 3000, 5000, and 7000 clumps per well), and initiate differentiation of each density on the same day.
12. Proceed to section II for differentiation.

II. DIFFERENTIATION OF hESCs/hiPSCs TO LUNG PROGENITOR CELLS

A. Protocol Diagram



B. Preparation of Media

Use sterile technique to prepare media for the differentiation protocol. There are four medium formulations required for the three stages of the protocol. Prepare Medium DE-1 and Medium DE-2 on Day 1, and Medium LP-1 and Medium LP-2 on Day 4, as indicated in the protocol in section C. Refer to Tables 1 and 2 for medium components, volumes, and preparation & storage. Volumes indicated are for one well; if preparing other volumes, adjust accordingly.

Day 1: Medium DE-1 and Medium DE-2

1. Thaw the entire bottle of STEMdiff™ Endoderm Basal Medium (Lung) at room temperature (15 - 25°C) or overnight at 2 - 8°C. Mix thoroughly.

   NOTE: If not using immediately, store at 2 - 8°C for up to 2 months. Alternatively, aliquot and store at -20°C. Do not exceed the shelf life of the medium. After thawing aliquots, use immediately or store at 2 - 8°C for up to 2 weeks. Do not re-freeze.
2. Warm 0.5 mL of STEMdiff™ Endoderm Basal Medium (Lung) to room temperature.
3. Thaw STEMdiff™ Definitive Endoderm Supplement MR and Supplement CJ on ice.
4. Prepare media by combining components as indicated in Table 1.

NOTE: Aliquot remaining supplements and store at -20°C. Do not exceed the expiry date as indicated on the label. After thawing the aliquots, use immediately. Do not re-freeze.

Table 1. Preparation of Medium DE-1 and Medium DE-2

MEDIUM	COMPONENT	VOLUME	PREPARATION & STORAGE
Medium DE-1 (0.5 mL)	STEMdiff™ Endoderm Basal Medium (Lung)	490 µL	Mix thoroughly and use immediately.
	STEMdiff™ Definitive Endoderm Supplement MR	5 µL	
	STEMdiff™ Definitive Endoderm Supplement CJ	5 µL	
Medium DE-2 (1 mL)	STEMdiff™ Endoderm Basal Medium (Lung)	990 µL	Mix thoroughly. Store at 2 - 8°C until required on Day 2 - 3.
	STEMdiff™ Definitive Endoderm Supplement CJ	10 µL	

Day 4: Medium LP-1 and Medium LP-2

1. Warm 3150 µL of STEMdiff™ Lung Basal Medium to room temperature.
2. Thaw STEMdiff™ Lung Supplement (10X) and STEMdiff™ Lung Progenitor Supplement 2 (100X) on ice. Thaw STEMdiff™ Lung Progenitor Supplement 1 (100X) at room temperature.
3. Prepare media by combining components as indicated in Table 2.

NOTE: Aliquot remaining supplements and store at -20°C. Do not exceed the expiry date as indicated on the label. After thawing the aliquots, use immediately. Do not re-freeze.

Table 2. Preparation of Medium LP-1 and Medium LP-2

MEDIUM	COMPONENT	VOLUME	PREPARATION & STORAGE
LP Basal Medium (3.5 mL)	STEMdiff™ Lung Basal Medium	3.15 mL	Mix thoroughly. If not using immediately, store at 2 - 8°C for up to 1 month.
	STEMdiff™ Lung Supplement (10X)	350 µL	
Medium LP-1 (1.5 mL)	LP Basal Medium	1.485 mL	Mix thoroughly. Divide into two aliquots: 1 mL/well for Day 4, and 0.5 mL/well for Day 5. Store the Day 5 aliquot at 2 - 8°C. Immediately before using the Day 4 aliquot, add 10 µM Y-27632 (Dihydrochloride) (section C step 10).
	STEMdiff™ Lung Progenitor Supplement 1 (100X)	15 µL	
Medium LP-2 (2 mL)	LP Basal Medium	1.98 mL	Mix thoroughly. Store at 2 - 8°C.
	STEMdiff™ Lung Progenitor Supplement 2 (100X)	20 µL	

C. Differentiation Protocol

Prior to initiating differentiation, assess confluency of hESCs/hiPSCs (prepared in section I) under a microscope after 24 hours of incubation. Cells should have < 5% differentiation and should be at least 70% confluent. If cells have not yet reached this level of confluency, replace medium with 0.5 - 1 mL of fresh mTeSR™1 or mTeSR™ Plus per well and incubate at 37°C for an additional 24 hours. Initial seeding densities for slow-proliferating cell lines may need to be optimized.

The following instructions are for a 24-well plate. Indicated volumes are for a single well. If using other cultureware, adjust volumes accordingly.

STAGE 1 (DAYS 1 - 3)

Day 1

1. Prepare Medium DE-1 and Medium DE-2 (section B). Warm Medium DE-1 to room temperature (15 - 25°C) and store Medium DE-2 at 2 - 8°C until required.
2. Aspirate mTeSR™1 or mTeSR™ Plus from hESCs/hiPSCs (prepared in section I). Add 0.5 mL of Medium DE-1 per well.
3. Incubate at 37°C with 5% CO_2 and 95% humidity for 24 hours.

Day 2

4. Warm a sufficient volume of Medium DE-2 (0.5 mL/well) at room temperature. Store the remaining Medium DE-2 at 2 - 8°C.
5. Aspirate medium from wells. Add 0.5 mL of Medium DE-2 per well.
6. Incubate at 37°C with 5% CO_2 and 95% humidity for 24 hours.

Day 3

7. Warm the remaining Medium DE-2 at room temperature.
8. Aspirate medium from wells. Add 0.5 mL of Medium DE-2 per well.
9. Incubate at 37°C with 5% CO_2 and 95% humidity for 24 hours. Proceed to Stage 2.

   IMPORTANT: Some cell death may be observed during Stage 1 culture. This does not affect the differentiation efficiency of the kit and has been accounted for in the recommended cell density of the protocol.

STAGE 2 (DAYS 4 - 6)

Day 4

10. Coat a 24-well tissue culture-treated plate(s) with Corning® Matrigel®. Prepare as many wells as required for replating.

11. Prepare Medium LP-2 and store at 2 - 8°C (section B). Prepare Medium LP-1 and divide into Day 4 and Day 5 aliquots as indicated in section B Table 2. Store the Day 5 aliquot at 2 - 8°C. Add 10 µM Y-27632 (Dihydrochloride) to the Day 4 aliquot (i.e. 2 µL of 5 mM Y-27632 [Dihydrochloride] stock solution + 1 mL Medium LP-1).

    NOTE: Typically, 1 well of Stage 1 can be replated into 6 - 12 wells of Stage 2. If replating into 6 wells, prepare 6.5 mL of Medium LP-1. Aliquot 3.5 mL into a separate tube, then add Y-27632 (Dihydrochloride). Use immediately for replating (step 13). Store the 3 mL aliquot (Day 5 aliquot) at 2 - 8°C.
12. Aspirate medium from wells of the Stage 1 culture. Add 0.25 mL of GCDR per well. Incubate at room temperature for 2 - 4 minutes.

    NOTE: Incubation times may vary when using different cell lines. Dissociation should be monitored under the microscope until the optimal time is determined.
13. Aspirate GCDR, then add 0.5 mL of Medium LP-1 + Y-27632 (Dihydrochloride) per well.
14. Dislodge monolayer into uniformly sized clumps of ~10 - 20 cells/clump by pipetting up and down gently with a 1 mL pipettor.
15. Transfer the detached cells into a 50 mL conical tube.
16. Add 0.5 mL of Medium LP-1 + Y-27632 (Dihydrochloride) per well to the Matrigel®-coated 24-well plate (prepared in step 10). Gently pipette the appropriate volume of cells into the wells containing medium.

    NOTE: If replating 1 well of Stage 1 into 6 wells of Stage 2, seed 83 µL of cells/well. There is a high cell line-to-cell line variability in the density of replating for Stage 1. An initial experiment is recommended to determine the optimal density for the cell line being used by seeding a range of split ratios (e.g. 1:6, 1:9, 1:12); typically, a lower density results in a higher NKX2.1 efficiency but lower cell yield.
17. Incubate at 37°C with 5% CO_2 and 95% humidity for 24 hours.

Day 5

18. Warm the Day 5 aliquot of Medium LP-1 to room temperature.
19. Aspirate medium from wells. Add 0.5 mL of Medium LP-1 per well.
20. Incubate at 37°C with 5% CO_2 and 95% humidity for 48 hours. Proceed to Stage 3.

STAGE 3 (DAYS 7 - 15)

Day 7

21. Aliquot a sufficient volume of Medium LP-2 for Day 7 use (0.5 mL/well) and warm to room temperature. Store the remaining Medium LP-2 at 2 - 8°C.
22. Aspirate medium from wells. Add 0.5 mL of Medium LP-2 per well.
23. Incubate at 37°C with 5% CO_2 and 95% humidity for 48 hours.

Day 9

24. Warm a sufficient volume of Medium LP-2 (0.5 mL/well) to room temperature.
25. Aspirate medium from wells. Add 0.5 mL of Medium LP-2 per well.
26. Incubate at 37°C with 5% CO_2 and 95% humidity for 48 hours.
27. Repeat steps 24 - 26 every other day until Day 15.

Day 15

Cells are ready to be assayed for the formation of proximal/distal lung cells or carried forward into more specialized assays.

Related Products

For related products, including specialized cell culture and storage media, supplements, antibodies, cytokines, and small molecules, visit www.stemcell.com, or contact us at techsupport@stemcell.com.