STEMdiff™ Branching Lung Organoid Kit

Serum-free media for generation and maturation of branching lung organoids from human ES and iPS cells

Catalog #100-0195 1 Kit
100-0528 1 Kit

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Product Description

STEMdiff™ Branching Lung Organoid Kit (Catalog #100-0195) is a serum-free medium system for efficient and reproducible generation of branching lung organoids from human embryonic stem (ES) and induced pluripotent stem (iPS) cells through four stages of differentiation: 1) definitive endoderm, 2) anterior foregut endoderm, 3) lung bud organoid, and 4) branching lung organoids. STEMdiff™ Branching Lung Organoid Kit has been optimized for differentiation of cells maintained in mTeSR™1 (Catalog #85850). Resulting organoids will develop proximal- and distal-like branching airway epithelial structures expressing EPCAM, NKX2.1, SOX2, SOX9, MUC1, and P63. When maintained beyond 28 days in a Matrigel® sandwich, there is an increase in expression levels of markers for more mature lung cells, such as SFTPC, SFTPB, and ABCA3. The kit provides sufficient media for culturing 12 Transwell® inserts of branching lung organoids for 28 days in a Matrigel® sandwich. For generating more organoids and for extended periods of culture, the components required for branching lung organoid maturation are available in STEMdiff™ Branching Lung Organoid Maturation Kit (Catalog #100-0528).

Product Information

All components listed below are sold as part of a kit (Catalog #100-0195 or 100-0528) and are not available for individual sale.

NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
STEMdiff™ Branching Lung Organoid Kit (100-0195)				
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™ Definitive Endoderm Supplement MR (100X)	05112	0.35 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
STEMdiff™ Definitive Endoderm Supplement CJ (100X)	05113	1.1 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.
STEMdiff™ Branching Lung Organoid Supplement A (100X)	100-0198	0.25 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
STEMdiff™ Branching Lung Organoid Supplement B (100X)**	100-0199	1.75 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
STEMdiff™ Branching Lung Organoid Maturation Kit (100-0528)				
STEMdiff™ Lung Basal Medium	100-0196	180 mL	Store at 2 - 8°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.
STEMdiff™ Branching Lung Organoid Supplement B (100X)**	100-0199	1.75 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.
**This component is light sensitive; minimize exposure to light when handling.

Materials Required But Not Included

PRODUCT NAME	CATALOG #
Costar® 24-Well Flat-Bottom Plate, Tissue Culture-Treated	38017
mTeSR™1	85850
Corning® Matrigel® hESC-Qualified Matrix	Corning 354277

PRODUCT NAME	CATALOG #
Gentle Cell Dissociation Reagent	100-0485
Falcon® 96-Well Flat-Bottom Microplate, Tissue Culture-Treated	38022
D-PBS (Without Ca++ and Mg++)	37350
Serological pipettes, 2 mL or 5 mL	e.g. 38002 or 38003
Conical tubes, 15 mL and 50 mL	e.g. 38009 and 38010
Greiner CELLSTAR® multiwell suspension culture plates, 24 wells	Sigma Aldrich M9312-100EA
Anti-Adherence Rinsing Solution	07010
DMEM/F-12 with 15 mM HEPES	36254
Costar® 6.5 mm Transwell®, 0.4 µm Pore Polyester Membrane Inserts	38024
Corning® Matrigel® Growth Factor Reduced (GFR) Basement Membrane Matrix, Phenol Red-free, LDEV-free	Corning 356231

Directions for Use

Please read the entire protocol before proceeding.

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. 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 Human ES/iPS Cells

II. Differentiation of Human ES/iPS Cells to Branching Lung Organoids (BLOs)

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

I. PASSAGING HUMAN ES/iPS CELLS

The following protocol is for clump passaging human ES or iPS cells cultured in mTeSR™1 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: Human ES and iPS cells are ready for passaging when cultures are approximately 70% confluent.

1. Coat a tissue culture-treated 24-well plate with Corning® Matrigel® (Corning Catalog #354277).
2. Prepare mTeSR™1 as directed in the applicable Product Information Sheet. Aliquot a sufficient volume of mTeSR™1 and warm to room temperature (15 - 25°C).
3. Use a microscope (4X magnification) to visually identify regions of differentiation in the ES/iPS 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 5 - 8 minutes.

   NOTE: Incubation times may vary when using different cell lines; dissociation should be monitored under the microscope until the optimal time is determined.
6. Aspirate GCDR and add 1 mL of mTeSR™1. Gently detach the colonies by scraping with a cell scraper/lifter.

   NOTE: Take care to minimize the breakup of colonies.
7. Using a 2 mL or 5 mL serological pipette, transfer the detached cell aggregates to a 50 mL conical tube.

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

   NOTE: Centrifugation of cell aggregates is not required.

8. Flick the 50 mL conical tube to break up the colonies evenly. Alternatively, use a 2 mL or 5 mL serological pipette to slowly pipette the cell clump mixture up and down. 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.

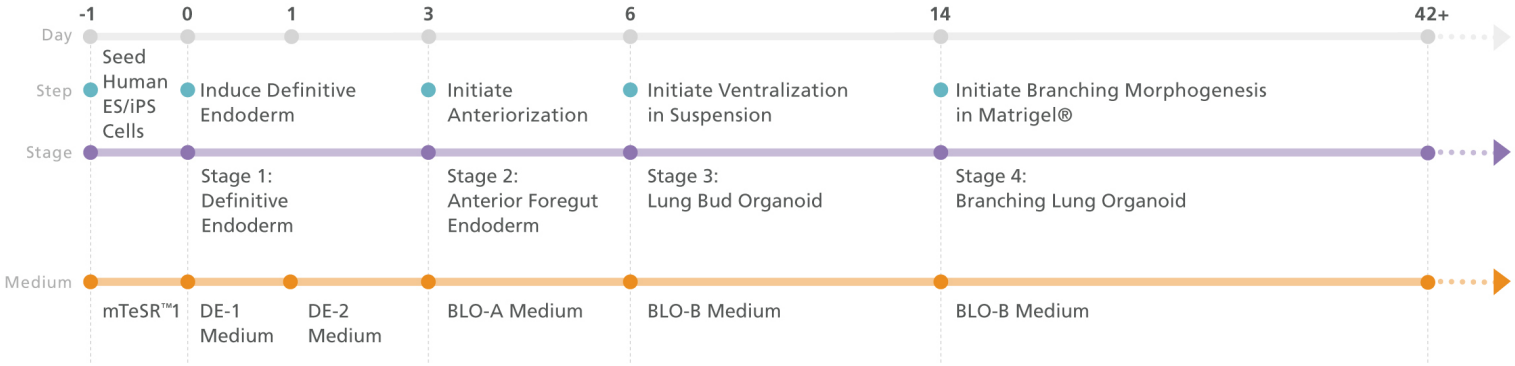
   NOTE: Counting clumps can be facilitated by scoring the bottom of the 96-well plate with a felt tip or lens marker. If most cell aggregates are > 200 µm in diameter, repeat steps 8 - 9.
10. Calculate the volume (in µL) of clump suspension required to seed 3000 clumps, as follows:

$$Volume\ of\ clump\ suspension\ (\mu L) = 3000\ cell\ clumps\ \div \frac{Number\ of\ clumps\ in\ 5\ \mu L}{5\ \mu L}$$

    NOTE: Optimal seeding density may vary when using different cell lines. An initial experiment may be required to determine the optimal clump seeding density for the cell line being used. Some cell lines may benefit from addition of 10 µM Y-27632 (Catalog #72302) at this stage.
11. Add the appropriate volume for 3000 clumps (calculated in step 10) to the coated 24-well plate (prepared in step 1) containing the appropriate volume of mTeSR™1 for a total of 1 mL per well. 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.
12. Proceed to section II for differentiation to BLOs.

II. DIFFERENTIATION OF HUMAN ES/iPS CELLS TO BRANCHING LUNG ORGANOIDS (BLOs)

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 four stages of the protocol. Prepare DE-1 Medium and DE-2 Medium on Day 0, BLO-A Medium on Day 3, and BLO-B Medium on Day 6, 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.

DE-1 Medium and DE-2 Medium (Day 0)

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 used 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. Thaw STEMdiff™ Definitive Endoderm Supplement MR and Supplement CJ on ice.
3. 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 aliquots, use immediately. Do not re-freeze.

Table 1. Preparation of DE-1 Medium and DE-2 Medium (Day 0)

MEDIUM	COMPONENT	VOLUME	PREPARATION & STORAGE
DE-1 Medium (0.5 mL)	STEMdiff™ Endoderm Basal Medium (Lung)	490 µL	Mix thoroughly. Warm to room temperature before use.
	STEMdiff™ Definitive Endoderm Supplement MR	5 µL	
	STEMdiff™ Definitive Endoderm Supplement CJ	5 µL	
DE-2 Medium (1 mL)	STEMdiff™ Endoderm Basal Medium (Lung)	990 µL	Mix thoroughly. Store at 2 - 8°C.
	STEMdiff™ Definitive Endoderm Supplement CJ	10 µL	

BLO-A Medium (Day 3) and BLO-B Medium (Day 6)

1. On **Day 3**, warm 13.5 mL of STEMdiff™ Lung Basal Medium to room temperature.
2. Thaw STEMdiff™ Lung Supplement (10X) and STEMdiff™ BLO Supplement A (100X) on ice. Mix each vial thoroughly.
   NOTE: There may be precipitate visible in STEMdiff™ BLO Supplement A; this will not affect performance.
3. Prepare BLO Basal Medium and BLO-A Medium by combining components as indicated in Table 2.
4. On **Day 6**, warm 13 mL of BLO Basal Medium at room temperature. Thaw STEMdiff™ BLO Supplement B on ice.
   NOTE: STEMdiff™ BLO Supplement B is light sensitive; minimize exposure to light when handling.
5. Prepare BLO-B Medium 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 aliquots, use immediately. Do not re-freeze.

Table 2. Preparation of BLO-A Medium (Day 3) and BLO-B Medium (Day 6)

MEDIUM	COMPONENT	VOLUME	PREPARATION & STORAGE
Day 3			
BLO Basal Medium (15 mL)	STEMdiff™ Lung Basal Medium	13.5 mL	Mix thoroughly. If not used immediately, store at 2 - 8°C for up to 2 months.
	STEMdiff™ Lung Supplement (10X)	1.5 mL	
BLO-A Medium (2 mL)	BLO Basal Medium	1.98 mL	Mix thoroughly. Store at 2 - 8°C.
	STEMdiff™ BLO Supplement A (100X)	20 µL	
Day 6			
BLO-B Medium (13 mL)	BLO Basal Medium	13 mL	Mix thoroughly. Store at 2 - 8°C.
	STEMdiff™ BLO Supplement B (100X)	130 µL	

C. Differentiation Protocol

Prior to initiating differentiation, assess confluency of human ES/iPS cells (prepared in section I) under a microscope after 24 hours of incubation. Cells should have < 5% differentiation and should be 30 - 60% confluent. If cells have not yet reached this level of confluency, replace medium with 0.5 - 1 mL of fresh mTeSR™1 per well, then 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 (DAY 0 - 3)

1. **Day 0**: Prepare DE-1 and DE-2 Media (see Preparation of Media). Warm DE-1 Medium to room temperature (15 - 25°C). Store DE-2 Medium at 2 - 8°C until required.
2. Aspirate mTeSR™1 from hPSCs (prepared in section I). Add 0.5 mL/well of room temperature DE-1 Medium.
3. Incubate at 37°C with 5% CO_2 and 95% humidity for 24 hours.

4. Day 1: Warm a sufficient volume of DE-2 Medium (0.5 mL/well) at room temperature. Store the remaining DE-2 Medium at 2 - 8°C.

   NOTE: It is recommended to feed the cells no later than 26 hours after the previous medium change.
5. Aspirate medium from wells and add 0.5 mL/well of DE-2 Medium down the side of the well of a tilted plate.
6. Incubate at 37°C with 5% CO_2 and 95% humidity for 24 hours.
7. Day 2: Warm the remaining DE-2 Medium at room temperature.
8. Aspirate medium from wells and add 0.5 mL/well of DE-2 Medium.
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 in culture during Stage 1. 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 (DAY 3 - 6)

10. Day 3: Prepare BLO-A Medium (see Preparation of Media). Warm BLO-A Medium to room temperature.
11. Aspirate medium from wells. Add 0.5 mL/well BLO-A Medium down the side of the well of a tilted plate.
12. Incubate at 37°C with 5% CO_2 and 95% humidity for 22 - 26 hours.
13. Day 4: Repeat steps 10 - 12.
14. Day 5: Repeat steps 10 - 12.

    NOTE: Free-floating anterior foregut endoderm (AFE) buds may appear on Day 5. However, for most cell lines it is not recommended to proceed to Stage 3 until Day 6.
15. Day 6: Warm BLO-A Medium to room temperature and proceed to Stage 3.

    NOTE: The monolayer may start peeling at this stage. As long as the monolayer is not aspirated, it will still produce AFE buds.

STAGE 3 (DAY 6 - 14)

16. Day 6: Prepare BLO-B Medium (see Preparation of Media). Warm the volume required (0.5 mL/well) to room temperature.
17. Coat a CELLSTAR® 24-well suspension culture plate with 0.25 mL/well Anti-Adherence Rinsing Solution. Rinse each well with sterile 0.5 mL DMEM/F-12 with 15 mM HEPES.
18. Using a 1 mL pipettor, transfer the free-floating AFE buds from each well of the monolayer plate into individual wells of the suspension culture plate prepared in step 17. Be careful not to aspirate the monolayer. Set the suspension plate aside while performing the next step.
19. Add 0.5 mL/well BLO-A Medium into the wells of the monolayer plate and incubate at 37°C with 5% CO_2 and 95% humidity for 22 - 26 hours.
20. Inspect the suspension plate under a microscope to assess the number of lifted AFE buds per well.

    NOTE: Counting AFE buds can be facilitated by scoring the bottom of the suspension culture plate with a felt tip or lens marker. It is recommended to pool the wells together if the number of AFE buds per well is < 30. If the total number of buds/well is > 50, aliquot into multiple wells, or discard extra buds, or cryopreserve buds using CryoStor® CS10 (Catalog #07930).
21. Perform a medium change as follows:
    a. Tilt the suspension plate containing AFE buds against an object (e.g. a tube rack). Once all of the AFE buds have settled to the bottom corner of the wells (approximately 2 minutes), slowly remove the medium, leaving behind a minimal amount of medium.
    b. Add 0.5 mL BLO-B Medium to each well. Incubate at 37°C with 5% CO_2 and 95% humidity for 22 - 26 hours.
22. Day 7: Warm BLO-B Medium to room temperature.
23. Using a 1 mL pipettor, transfer the free-floating AFE buds from each well of the monolayer plate into individual wells of the suspension plate. Discard the monolayer plate.

    NOTE: There may be buds still attached to the monolayer. The monolayer may be washed, but the resulting bud suspension may contain undesirable cells. We recommend proceeding only with free-floating AFE buds.
24. Inspect the suspension plate under a microscope to assess the number of AFE buds per well. Incubate at 37°C with 5% CO_2 and 95% humidity.

    NOTE: Counting AFE buds can be facilitated by scoring the bottom of the suspension culture plate with a felt tip or lens marker.
25. It is recommended to pool the wells together if the number of AFE buds per well is < 30. If the total number of buds/well is > 50, aliquot into multiple wells (using an additional coated suspension culture plate [prepared as described in step 17] if necessary), or discard extra buds, or cryopreserve buds using CryoStor® CS10. Perform a medium change every 2 days by removing medium then adding fresh BLO-B Medium (as described in step 21). This will mature the AFE buds into lung bud organoids (LBOs).

    NOTE: Once per week, a medium change can be performed after 3 days.

26. After 7 days of incubation, proceed to Stage 4.

    NOTE: Some organoid death and fusion is expected at this point. Organoids that survive will grow significantly in size and begin to develop thicker epithelial layers, demonstrating internal structures. Organoids may be left in suspension up to an additional 7 days (Day 21) before proceeding to Stage 4.

STAGE 4 (DAY 14 - 42+)

27. Prepare the Matrigel® sandwich insert by transferring a sterile Transwell® insert into a sterile tissue culture-treated flat-bottom 24-well plate.

    NOTE: Between 1 - 5 LBOs may be embedded in a single insert. Unused Transwell® inserts can be stored in a sterile flat-bottom 24-well plate secured with Parafilm® for future use.

28. Thaw 130 µL of Matrigel® (Corning Catalog #356231) on ice. Place a 15 mL conical tube on ice.
29. Warm BLO-B Medium to room temperature.
30. Using a sterile wide bore 200 µL pipette tip, transfer 1 - 5 LBOs in 50 - 200 µL of medium into the cooled 15 mL conical tube. Keep on ice and wait for LBOs to settle to the bottom of the tube.
31. Prepare the Matrigel® sandwich as follows:
    a. Add 50 µL of thawed Matrigel® to the center of the insert to be used. Incubate at room temperature for at least 10 minutes.
    b. Using a sterile 200 µL pipette tip, remove as much medium as possible from the LBOs.
    c. Immediately add 30 µL of Matrigel® to the LBOs. Keep on ice.
    d. Using a sterile wide bore 200 µL pipette tip, transfer 30 µL of LBO-containing Matrigel® to the top and center of the solidified Matrigel® layer in the insert.
    e. Incubate at room temperature for 5 - 10 minutes.
    f. Layer 50 µL of thawed Matrigel® on top of the embedded LBOs. Incubate at 37°C for 15 minutes.
32. Add 0.5 mL room temperature BLO-B Medium to the basal chamber and 0.3 mL to the apical chamber. Incubate at 37°C with 5% CO_2 and 95% humidity.
33. Perform a medium change every 2 days by removing medium from both the apical and basal chambers then adding fresh room temperature BLO-B Medium as described in step 29. Incubate at 37°C with 5% CO_2 and 95% humidity.

    NOTE: Once per week, a medium change can be performed after 3 days to accommodate for the weekend.

    NOTE: LBOs should start to bud and express the lung progenitor marker NKX2.1 within 2 weeks in the Matrigel® sandwich and mature into BLOs by week 4.

34. BLOs can be matured further and maintained for at least 3 months, topping up with additional Matrigel® and BLO-B Medium. Layer 50 µL of fresh thawed Matrigel® on top of BLOs every 4 weeks. STEMdiff™ Branching Lung Organoid Maturation Kit (Catalog #100-0528) is available if additional BLO-B Medium is required for maturation of organoids.

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.