STEMdiff™ Definitive Endoderm Kit

Defined animal component-free medium for the differentiation of human ESCs and iPSCs to definitive endoderm

Catalog #05110 1 Kit
Catalog #05115 1 Kit

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Product Description

STEMdiff™ Definitive Endoderm Kit is a complete, serum- and animal component-free kit that supports highly efficient differentiation of human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) to definitive endoderm cells. Cells differentiated using STEMdiff™ Definitive Endoderm Kit express high levels of endoderm markers, including CD184 (CXCR4), SOX17, FOXA2, and CD117 (c-KIT), and lack expression of markers of ectoderm, mesoderm, and the undifferentiated state. Definitive endoderm cells generated using this kit are multipotent and capable of further differentiation towards pancreatic, intestinal, pulmonary, and hepatic cell lineages, thus providing a robust tool for developmental studies, disease modeling, and drug discovery.

STEMdiff™ Definitive Endoderm Kit (Catalog #05110) is optimized for the differentiation of hESCs and hiPSCs (such as Healthy Control Human iPSC Line, Female, SCTi003-A [Catalog #200-0511]) cultured in eTeSR™ (Catalog #100-1215), mTeSR™1 (Catalog #85850), mTeSR™ Plus (Catalog #100-0276), and TeSR™-AOF (Catalog #100-0401).

STEMdiff™ Definitive Endoderm Kit (TeSR™-E8™ Optimized; Catalog #05115) is optimized for the differentiation of hESCs and hiPSCs cultured in TeSR™-E8™ (Catalog #05990).

Ordering Information

PRODUCT NAME	CATALOG #	SIZE	COMPONENTS
STEMdiff™ Definitive Endoderm Kit	05110	1 Kit	• STEMdiff™ Endoderm Basal Medium • STEMdiff™ Definitive Endoderm Supplement MR (100X) • STEMdiff™ Definitive Endoderm Supplement CJ (100X)
STEMdiff™ Definitive Endoderm Kit (TeSR™-E8™ Optimized)	05115	1 Kit	• STEMdiff™ Endoderm Basal Medium • STEMdiff™ Definitive Endoderm Supplement MR (100X) • STEMdiff™ Definitive Endoderm Supplement CJ (100X) • STEMdiff™ Definitive Endoderm TeSR™-E8™ Supplement (20X)

Component Storage and Stability

The following components are sold as part of complete kits (Catalog #05110 and 05115) and are not available for individual sale.

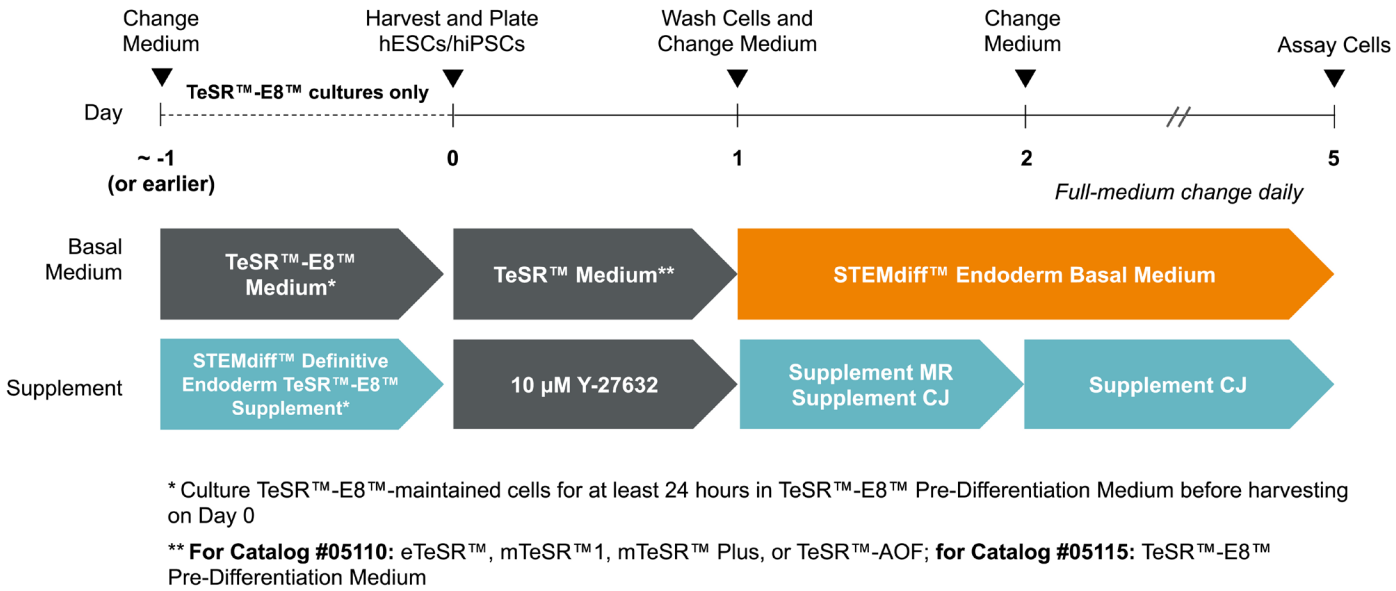
COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
STEMdiff™ Endoderm Basal Medium	05111	100 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
STEMdiff™ Definitive Endoderm Supplement MR (100X)	05112	0.35 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
STEMdiff™ Definitive Endoderm Supplement CJ (100X)	05113	1.1 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
STEMdiff™ Definitive Endoderm TeSR™-E8™ Supplement (20X)	05116	7 mL	Store at 2 - 8°C.	Stable for 12 months from date of manufacture (MFG) on label.

Materials Required but Not Included

PRODUCT NAME	CATALOG #
CellAdhere™ Laminin-521 OR Corning® Matrigel® hESC-qualified matrix OR Vitronectin XF™*	200-0117 OR Corning 354277 OR 07180
Costar® 6-Well Flat-Bottom Plate, Tissue Culture-Treated** OR 6-Well Flat-Bottom Plate, Non-Treated†	38015 OR 38040
DMEM/F-12 with 15 mM HEPES	36254
D-PBS (Without Ca++ and Mg++)	37350
Gentle Cell Dissociation Reagent	100-0485
Hausser Scientific™ Bright-Line Hemocytometer	100-1181
eTeSR™‡ OR mTeSR™1‡ OR mTeSR™ Plus‡ OR TeSR™-AOF‡ OR TeSR™-E8™§	100-1215 OR 85850 OR 100-0276 OR 100-0401 OR 05990
Trypan Blue	07050
Y-27632 (Dihydrochloride)	72302

* If using Vitronectin XF™, CellAdhere™ Dilution Buffer (Catalog #07183) is also required.
** For use with Corning® Matrigel® or CellAdhere™ Laminin-521
† For use with Vitronectin XF™
‡ For use with STEMdiff™ Definitive Endoderm Kit (Catalog #05110)
§ For use with STEMdiff™ Definitive Endoderm Kit (TeSR™-E8™ Optimized; Catalog #05115)

Protocol Diagram



* Culture TeSR™-E8™-maintained cells for at least 24 hours in TeSR™-E8™ Pre-Differentiation Medium before harvesting on Day 0

** **For Catalog #05110:** eTeSR™, mTeSR™1, mTeSR™ Plus, or TeSR™-AOF; **for Catalog #05115:** TeSR™-E8™ Pre-Differentiation Medium

Figure 1. Protocol Diagram for the Differentiation of Human Pluripotent Stem Cells to Definitive Endoderm Cells Using STEMdiff™ Definitive Endoderm Kit or STEMdiff™ Definitive Endoderm Kit (TeSR™-E8™ Optimized)

Preparation of Media and Reagents

A. DEFINITIVE ENDODERM MEDIA

Use sterile technique to prepare Definitive Endoderm Medium 1 (STEMdiff™ Endoderm Basal Medium + STEMdiff™ Definitive Endoderm Supplement MR [100X] + STEMdiff™ Definitive Endoderm Supplement CJ [100X]) and Definitive Endoderm Medium 2 (STEMdiff™ Endoderm Basal Medium + STEMdiff™ Definitive Endoderm Supplement CJ [100X]). Prepare each medium as needed in Directions for Use. Refer to Table 1 for medium components, volumes, and in-use storage and stability.

1. Thaw the entire bottle of STEMdiff™ Endoderm Basal Medium 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 until 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. Do not re-freeze.
2. Thaw supplement(s) on ice or overnight at 2 - 8°C. Mix thoroughly and keep on ice until use.

   NOTE: If not using immediately, aliquot 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.
3. Prepare media as indicated in Table 1. Mix thoroughly. Warm medium to room temperature before use. If preparing other volumes, adjust accordingly.

Table 1. Preparation of Media

MEDIUM	COMPONENT	VOLUME	IN-USE STORAGE AND STABILITY
Definitive Endoderm Medium 1 (10 mL)	STEMdiff™ Endoderm Basal Medium	9.8 mL	Prepare fresh before use. Do not store.
	STEMdiff™ Definitive Endoderm Supplement MR (100X)	100 µL	
	STEMdiff™ Definitive Endoderm Supplement CJ (100X)	100 µL	
Definitive Endoderm Medium 2 (10 mL)	STEMdiff™ Endoderm Basal Medium	9.9 mL	Prepare fresh before use. Store at 2 - 8°C between medium changes.
	STEMdiff™ Definitive Endoderm Supplement CJ (100X)	100 µL	
TeSR™-E8™ Pre-Differentiation Medium* (20 mL)	Complete TeSR™-E8™ medium	19 mL	Store at 2 - 8°C for up to 2 weeks.
	STEMdiff™ Definitive Endoderm TeSR™-E8™ Supplement	1 mL	

* For differentiation of hESCs/hiPSCs cultured in TeSR™-E8™ using STEMdiff™ Definitive Endoderm Kit (TeSR™-E8™ Optimized; Catalog #05115) only.

B. COATING PLATES WITH CELLADHERE™ LAMININ-521, CORNING® MATRIGEL®, OR VITRONECTIN XF™

For complete instructions on coating plates with CellAdhere™ Laminin-521, Corning® Matrigel®, or Vitronectin XF™, refer to the Technical Manual for eTeSR™, mTeSR™1, mTeSR™ Plus, TeSR™-AOF, or TeSR™-E8™, available at www.stemcell.com.

Matrix-coated plates should be prepared in advance and be brought to room temperature (15 - 25°C) for at least 30 minutes prior to use.

Directions for Use

Please read the entire protocol before proceeding. Use sterile technique when performing the following protocols:

A. Passaging Cells for Definitive Endoderm Induction (Day 0)

B. Differentiating Monolayer Cultures to Definitive Endoderm (Days 1 - 5)

A. PASSAGING CELLS FOR DEFINITIVE ENDODERM INDUCTION (DAY 0)

The following instructions are for harvesting hESCs or hiPSCs maintained in eTeSR™, mTeSR™1, mTeSR™ Plus, TeSR™-AOF, or TeSR™-E8™ from one well of a 6-well plate and seeding into another 6-well plate. If using other cultureware, adjust volumes accordingly.

NOTE: hESCs and hiPSCs are ready to harvest when cultures are approximately 70% confluent.

eTeSR™, mTeSR™1, mTeSR™ Plus, or TeSR™-AOF Cultures

NOTE: For complete instructions on maintaining high-quality hESCs and hiPSCs for use in differentiation, refer to the Technical Manual for eTeSR™, mTeSR™1, mTeSR™ Plus, or TeSR™-AOF, available at www.stemcell.com.

DAY 0

1. Coat a 6-well plate with CellAdhere™ Laminin-521, Corning® Matrigel®, or Vitronectin XF™ (Preparation section B). Warm to room temperature (15 - 25°C) before use.

   NOTE: If using CellAdhere™ Laminin-521 or Corning® Matrigel®, use tissue culture-treated plates; if using Vitronectin XF™, use non-tissue culture-treated plates.
2. Warm sufficient volumes of eTeSR™, mTeSR™1, mTeSR™ Plus, or TeSR™-AOF, and DMEM/F-12 with 15 mM HEPES to room temperature.
3. Prepare Single-Cell Passaging Medium by adding Y-27632 (Dihydrochloride) to eTeSR™, mTeSR™1, mTeSR™ Plus, or TeSR™-AOF to a final concentration of 10 µM.
4. Passage cells as follows:
   a. Wash the well to be passaged with 1 mL of D-PBS (Without Ca++ and Mg++).
   b. Remove and discard the D-PBS wash. Add 1 mL of Gentle Cell Dissociation Reagent to the well.
   c. Incubate the plate at 37°C for 8 - 10 minutes.
   d. Dislodge cells by pipetting up and down 1 - 3 times using a pipettor with a 1 mL tip. Ensure all remaining cell aggregates are broken up into single cells.
   e. Immediately transfer the cells to a conical tube containing an equal volume of DMEM/F-12 with 15 mM HEPES. Wash the well once with 1 mL of DMEM/F-12 with 15 mM HEPES to collect any remaining cells and transfer to the same tube.
   f. Centrifuge the tube at 300 x g for 5 minutes.
   g. Resuspend the cells in 1 mL of Single-Cell Passaging Medium (prepared in step 3) and count the number of live cells using Trypan Blue and a Hausser Scientific™ Bright-Line Hemocytometer.
   h. Plate the cells at a density of 2.1 x 10^5 cells/cm^2 (i.e. 2 x 10^6 cells/well) onto the coated 6-well plate (prepared in step 1). If necessary, adjust the seeding density so that the cells are approximately 90 - 100% confluent on Day 1.
5. Incubate the plate at 37°C for 24 hours.
6. Continue to section B for differentiation.

TeSR™-E8™ Cultures

For complete instructions on preparing complete TeSR™-E8™ medium and for passaging hESCs and hiPSCs maintained in TeSR™-E8™, refer to the Technical Manual: Maintenance of Human Pluripotent Stem Cells in TeSR™-E8™, available at www.stemcell.com.

1. Follow a standard passaging protocol to passage TeSR™-E8™ cultures into one well of a 6-well plate coated with Corning® Matrigel® or Vitronectin XF™. Perform daily full-medium changes with complete TeSR™-E8™ medium for 4 - 5 days.
2. Prepare a sufficient volume of complete TeSR™-E8™ Pre-Differentiation Medium (Preparation section A) to be used until step 5 (i.e. at least 4 mL per well).

   NOTE: Complete TeSR™-E8™ Pre-Differentiation Medium can be stored at 2 - 8°C for up to 2 weeks.
3. Aliquot and warm a sufficient volume (2 mL/well) of complete TeSR™-E8™ Pre-Differentiation Medium to room temperature.
4. Aspirate medium from the well and add 2 mL of complete TeSR™-E8™ Pre-Differentiation Medium.
5. Incubate at 37°C for 24 hours. Perform daily full-medium changes using complete TeSR™-E8™ Pre-Differentiation Medium (steps 3 and 4) until cultures are approximately 70% confluent before passaging.

   NOTE: For optimal differentiation performance, cells must be exposed to complete TeSR™-E8™ Pre-Differentiation Medium for at least 24 hours before passaging on Day 0.

DAY 0

6. Coat a 6-well plate with Corning® Matrigel® or Vitronectin XF™. Warm to room temperature (15 - 25°C) before use.
7. Warm sufficient volumes of complete TeSR™-E8™ Pre-Differentiation Medium and DMEM/F-12 with 15 mM HEPES to room temperature.
8. Prepare Single-Cell Passaging Medium by adding Y-27632 (Dihydrochloride) to complete TeSR™-E8™ Pre-Differentiation Medium to a final concentration of 10 µM.
9. Passage cells as follows:
   a. Wash the well to be passaged with 1 mL of D-PBS (Without Ca++ and Mg++).
   b. Remove and discard the D-PBS wash. Add 1 mL of Gentle Cell Dissociation Reagent to the well.

c. Incubate the plate at 37°C for 8 - 10 minutes.
d. Dislodge cells by pipetting up and down 1 - 3 times using a pipettor with a 1 mL tip. Ensure all remaining cell aggregates are broken up into single cells.
e. Immediately transfer the cells to a conical tube containing an equal volume of DMEM/F-12 with 15 mM HEPES. Wash the well once with 1 mL of DMEM/F-12 with 15 mM HEPES to collect any remaining cells and transfer to the same tube.
f. Centrifuge the tube at 300 x g for 5 minutes.
g. Resuspend the cells in 1 mL of Single-Cell Passaging Medium (prepared in step 8) and count the number of live cells using a hemocytometer.
h. Plate the cells at a density of 2.1 x 10^5 cells/cm^2 (i.e. 2 x 10^6 cells/well) onto the coated 6-well plate (prepared in step 6). If necessary, adjust the seeding density so that the cells are approximately 90 - 100% confluent on Day 1.

10. Incubate at 37°C for 24 hours.
11. Continue to section B for differentiation.

B. DIFFERENTIATING MONOLAYER CULTURES TO DEFINITIVE ENDODERM (DAYS 1 - 5)

The following instructions are for differentiating Day 1 hESCs or hiPSCs in a 6-well plate. If using other cultureware, adjust volumes accordingly.

DAY 1

1. Prepare a sufficient volume of Definitive Endoderm Medium 1 (Preparation section A) for use on Day 1 and warm to room temperature (15 - 25°C). Aliquot a sufficient volume of DMEM/F-12 with 15 mM HEPES and warm to room temperature.
2. Remove and discard spent medium from each well. Wash the cells with 1 mL/well of DMEM/F-12 with 15 mM HEPES.
3. Discard the wash and add 2 mL/well of Definitive Endoderm Medium 1.
4. Incubate at 37°C for 24 hours.

DAYS 2 - 4

5. Prepare a sufficient volume of Definitive Endoderm Medium 2 (see Preparation section A) for use on Days 2, 3, and 4 (i.e. 2 mL/well x 3 days = 6 mL total per well). Store at 2 - 8°C.
6. Aliquot and warm (37°C) a sufficient volume (i.e. 2 mL per well) of Definitive Endoderm Medium 2.
7. Remove and discard spent medium. Add 2 mL/well of Definitive Endoderm Medium 2.
   NOTE: A wash with DMEM/F-12 with 15 mM HEPES is not required at this step or during subsequent medium changes.
8. Incubate at 37°C for 24 hours.
9. Perform daily full-medium changes (steps 6 - 8) with Definitive Endoderm Medium 2 on Days 3 and 4.

DAY 5

Cells are ready to be assayed for the formation of definitive endoderm or carried forward into more specialized lineage differentiation protocols.

NOTE: Expression of definitive endoderm markers may peak by Day 4 in some cell lines.

Assessment of Definitive Endoderm Cells

Purity of definitive endoderm cells can be measured by flow cytometry after labeling with fluorochrome-conjugated anti-CXCR4 (e.g. Anti-Human CD184 [CXCR4] Antibody, Clone 12G5; Catalog #60089) and anti-c-Kit (e.g. Anti-Human CD117 [c-Kit] Antibody, Clone 104D2; Catalog #60087) or anti-SOX17 antibodies. Results may vary depending on cell line used.

Related Products

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