STEMdiff™ Definitive Endoderm Kit

Defined animal component-free medium for the differentiation of human ES and iPS cells to definitive endoderm

Catalog #05110 1 Kit
#05115 1 Kit

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Product Description

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

STEMdiff™ Definitive Endoderm Kit (Catalog #05110) and STEMdiff™ Definitive Endoderm Kit (TeSR™-E8™ Optimized; Catalog #05115) have been optimized for the differentiation of human ES and iPS cells cultured in mTeSR™1 or TeSR™-E8™, respectively.

Product Information

PRODUCT NAME	CATALOG #	SIZE	COMPONENTS
STEMdiff™ Definitive Endoderm Kit	05110	1 Kit	• STEMdiff™ Definitive Endoderm Basal Medium (100 mL) • STEMdiff™ Definitive Endoderm Supplement A (100X; 0.35 mL) • STEMdiff™ Definitive Endoderm Supplement B (100X; 1.1 mL)
STEMdiff™ Definitive Endoderm Kit (TeSR™-E8™ Optimized)	05115	1 Kit	• STEMdiff™ Definitive Endoderm Basal Medium (100 mL) • STEMdiff™ Definitive Endoderm Supplement A (100X; 0.35 mL) • STEMdiff™ Definitive Endoderm Supplement B (100X; 1.1 mL) • STEMdiff™ Definitive Endoderm TeSR™-E8™ Supplement (20X; 7 mL)

Component Storage and Stability

The following components are sold as part of the STEMdiff™ Definitive Endoderm Kits (see Product Information) and are not available for individual sale.

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

Handling Frozen Components

05111 STEMdiff™ Definitive Endoderm Basal Medium

- Thaw entire bottle at room temperature (15 - 25°C) or overnight at 2 - 8°C, and mix thoroughly. Once thawed, use immediately or 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.

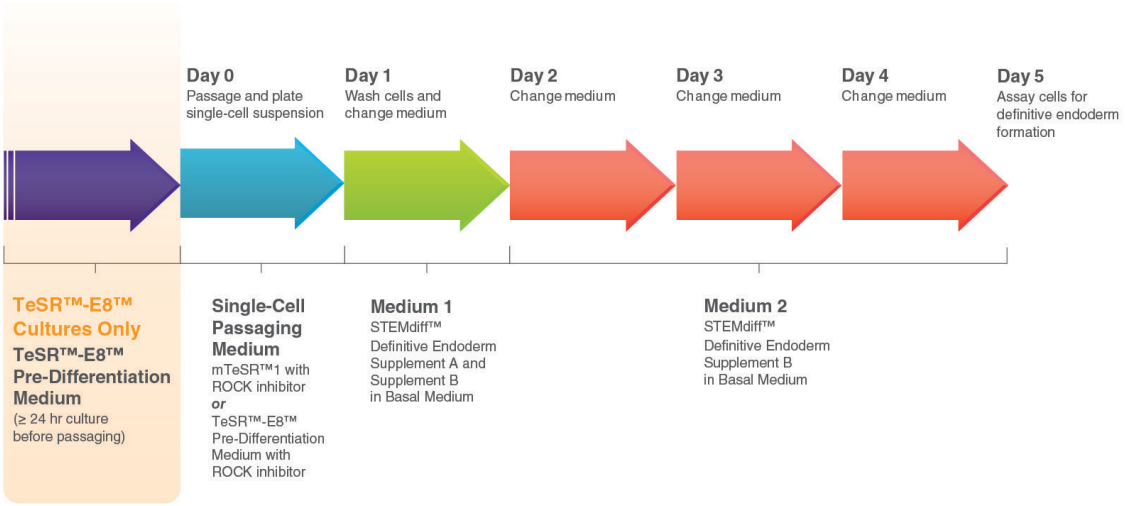
05112 STEMdiff™ Definitive Endoderm Supplement A (100X) OR **05113 STEMdiff™ Definitive Endoderm Supplement B (100X)**

- Thaw on ice and mix thoroughly. Once thawed, use immediately or aliquot and store at -20°C for up to 12 months from the date of manufacture as indicated on the label. After thawing the aliquots, use immediately. Do not re-freeze.

Materials Required But Not Included

PRODUCT NAME	CATALOG #
mTeSR™1 OR TeSR™-E8™	85850 OR 05940
Corning® Matrigel® hESC-qualified matrix OR Vitronectin XF™	Corning 354277 OR 07180
DMEM/F-12 with 15 mM HEPES	36254
Gentle Cell Dissociation Reagent	07174
D-PBS (Without Ca++ and Mg++)	37350
Y-27632	72302

Schematic of STEMdiff™ Definitive Endoderm Kit Procedure



Directions for Use

Please read the entire protocol before proceeding.

NOTE: For complete instructions on coating plates with Corning® Matrigel® or Vitronectin XF™, and maintaining high quality human ES and iPS cells for use in differentiation, please refer to the Technical Manual: Maintenance of Human Pluripotent Stem Cells in mTeSR™1 (Document #28315) or TeSR™-E8™ (Document #29267), available at www.stemcell.com or contact us to request a copy. 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.

Use sterile techniques when performing the following protocols. The following are instructions for use with 6-well plates. Indicated volumes are for a single well. If using alternative cultureware, adjust volumes accordingly.

1. Passaging Cells for Definitive Endoderm Induction

For optimal product performance, passage human ES or iPS cells using the specific passaging protocols for cells cultured in mTeSR™1 or TeSR™-E8™ as outlined in this section, before proceeding with differentiation to definitive endoderm (section 2).

NOTE: Human ES and iPS cells are ready for passage when cultures are approximately 70% confluent.

mTeSR™1 Cultures

This protocol is specific to human ES and iPS cells cultured in mTeSR™1 medium.

1. On Day 0, warm (15 - 25°C) sufficient volumes of mTeSR™1, DMEM/F-12, and Gentle Cell Dissociation Reagent for passaging. Prepare Single-Cell Passaging Medium by adding Y-27632 to mTeSR™1 to reach a final concentration of 10 µM.
2. Wash the well to be passaged with 1 mL of D-PBS (Without Ca++ and Mg++).
3. Aspirate wash medium and add 1 mL of Gentle Cell Dissociation Reagent.
4. Incubate at 37°C for 8 - 10 minutes.
5. 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.
6. Immediately transfer cells to a tube containing an equal volume of DMEM/F-12. Wash the 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.
7. Resuspend cells in 1 mL of Single-Cell Passaging Medium and count the number of live cells using a hemocytometer.
8. Plate cells at a density of 2.1 x 10^5 cells/cm^2 (i.e. 2 x 10^6 cells/well) onto pre-coated plates. Adjust density if necessary, so that the cells are approximately 90 - 100% confluent on Day 1.
9. Incubate at 37°C for 24 hours.
10. Continue to section 2 (Differentiating Monolayer Cultures to Definitive Endoderm).

TeSR™-E8™ Cultures

This protocol is specific to human ES and iPS cells cultured in TeSR™-E8™ medium.

1. Follow a standard passaging protocol to passage TeSR™-E8™ cultures into one well of a 6-well plate, and perform daily medium changes for 4 days.

   NOTE: Refer to the Technical Manual: Maintenance of Human Pluripotent Stem Cells in TeSR™-E8™ (Document #29267) for recommended passaging protocols using TeSR™-E8™.
2. Four days after passaging TeSR™-E8™ cultures, prepare complete TeSR™-E8™ Pre-Differentiation Medium by diluting cold (2 - 8°C) STEMdiff™ Definitive Endoderm TeSR™-E8™ Supplement 1 in 20 in cold (2 - 8°C) TeSR™-E8™ medium (e.g. add 1 mL of Supplement to 19 mL of TeSR™-E8™). Prepare sufficient complete TeSR™-E8™ Pre-Differentiation Medium to be used until step 6 (i.e. at least 4 mL per well).

   NOTE: Complete Pre-Differentiation Medium can be stored at 2 - 8°C for up to 2 weeks.
3. Warm (15 - 25°C) only the volume of complete TeSR™-E8™ Pre-Differentiation Medium required on this day (i.e. 2 mL per well). Store remaining medium at 2 - 8°C.
4. Aspirate medium from the culture well and add 2 mL of complete TeSR™-E8™ Pre-Differentiation Medium.
5. Incubate at 37°C and perform daily medium changes (steps 3 and 4) until cultures are approximately 70% confluent, and are ready to be passaged.

   NOTE: For optimal differentiation performance, cells must be exposed to complete TeSR™-E8™ Pre-Differentiation Medium for at least 24 hours before the next passaging step.
6. Passage cells (Day 0):
   i. Warm (15 - 25°C) sufficient volumes of complete TeSR™-E8™ Pre-Differentiation Medium, DMEM/F-12, and Gentle Cell Dissociation Reagent for passaging. Prepare Single-Cell Passaging Medium by adding Y-27632 to TeSR™-E8™ Pre-Differentiation Medium to reach a final concentration of 10 µM.
   ii. Wash the well to be passaged with 1 mL of D-PBS (Without Ca++ and Mg++).
   iii. Aspirate wash medium and add 1 mL of Gentle Cell Dissociation Reagent.
   iv. Incubate at 37°C for 8 - 10 minutes.
   v. 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.
   vi. Immediately transfer cells to a tube containing an equal volume of DMEM/F-12. Wash the 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.
   vii. Resuspend cells in 1 mL of Single-Cell Passaging Medium and count the number of live cells using a hemocytometer.

viii. Plate cells at a density of 2.1 x 10^5 cells/cm^2 (i.e. 2 x 10^6 cells/well) onto pre-coated plates. Adjust density if necessary, so that the cells are approximately 90 - 100% confluent on Day 1.

ix. Incubate at 37°C for 24 hours.

x. Continue to section 2 (Differentiating Monolayer Cultures to Definitive Endoderm).

2. Differentiating Monolayer Cultures to Definitive Endoderm

1. On Day 1, warm (37°C) sufficient volumes of DMEM/F-12 and STEMdiff™ Definitive Endoderm Basal Medium for Day 1 use.
2. Prepare Medium 1 by diluting both STEMdiff™ Definitive Endoderm Supplement A and STEMdiff™ Definitive Endoderm Supplement B 1 in 100 in STEMdiff™ Definitive Endoderm Basal Medium (e.g. add 10 µL of Supplement A and 10 µL of Supplement B to 980 µL of Basal Medium).

   NOTE: Supplements should be thawed on ice and kept cold until added to STEMdiff™ Definitive Endoderm Basal Medium.
3. Aspirate medium and wash with 1 mL DMEM/F-12.
4. Aspirate wash medium and replace with 2 mL of Medium 1.
5. Incubate at 37°C for 24 hours.
6. On Day 2, prepare Medium 2 by diluting STEMdiff™ Definitive Endoderm Supplement B 1 in 100 in STEMdiff™ Definitive Endoderm Basal Medium (e.g. add 10 µL of Supplement B to 990 µL of Basal Medium). Prepare sufficient Medium 2 to be used on Days 2, 3 and 4 (i.e. 6 mL per well).

   NOTE: STEMdiff™ Definitive Endoderm Supplement B should be thawed on ice and added to cold (2 - 8°C) STEMdiff™ Definitive Endoderm Basal Medium.
7. Warm (37°C) only the volume of Medium 2 required for Day 2 use (i.e. 2 mL per well). Store remaining Medium 2 at 2 - 8°C.
8. Aspirate medium from the well and add 2 mL of Medium 2.

   NOTE: A wash step with DMEM/F-12 is not required at this step or during subsequent medium changes.
9. Incubate at 37°C for 24 hours.
10. On Day 3, warm (37°C) only the volume of Medium 2 required for Day 3 use (i.e. 2 mL per well). Store remaining Medium 2 at 2 - 8°C.
11. Aspirate medium from the well and add 2 mL of Medium 2.
12. Incubate at 37°C for 24 hours.
13. On Day 4, warm (37°C) only the volume of Medium 2 required for the Day 4 medium change (i.e. 2 mL per well).
14. Aspirate medium from the well and add 2 mL of Medium 2.
15. Incubate at 37°C for 24 hours.
16. On 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

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