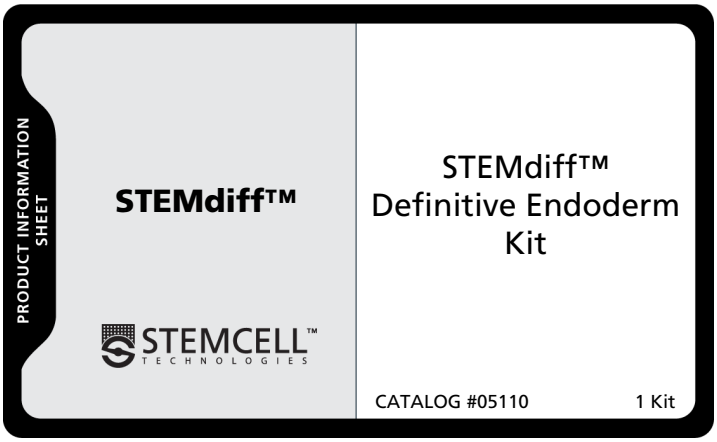
PRODUCT DESCRIPTION

STEMdiff™ Definitive Endoderm Kit is a serum-free and animal component-free combination of basal medium and supplements for the differentiation of human embryonic stem (ES) cells and induced pluripotent stem (iPS) cells to definitive endoderm. Cells differentiated to definitive endoderm using this kit express high levels of CXCR4 and SOX17 and can be used to generate more specified cells of endodermal lineage including hepatocytes and pancreatic precursors.



COMPONENTS

05111 STEMdiff™ Definitive Endoderm Basal Medium 100 mL

05112 STEMdiff™ Definitive Endoderm Supplement A (100X) 0.35 mL

05113 STEMdiff™ Definitive Endoderm Supplement B (100X) 1.1 mL

These products have been aseptically manufactured using tightly controlled processes and are sterility tested.

STABILITY AND STORAGE

05111 STEMdiff™ Definitive Endoderm Basal Medium 100 mL

Product stable at -20°C for 12 months from date of manufacture as indicated on label. Thaw entire bottle at room temperature (15 - 25°C). Once thawed, medium is stable for 2 months at 2 - 8°C. Thawed medium can be aliquoted and stored at -20°C. Avoid additional freeze-thaw cycles.

05112 STEMdiff™ Definitive Endoderm Supplement A (100X) 0.35 mL

Product stable at -20°C for 12 months from date of manufacture as indicated on label. Supplement should be thawed on ice, aliquoted and stored at -20°C. Avoid additional freeze-thaw cycles. Product is sensitive to light and should be kept in the dark whenever possible.

05113 STEMdiff™ Definitive Endoderm Supplement B (100X) 1.1 mL

Product stable at -20°C for 12 months from date of manufacture as indicated on label. Supplement should be thawed on ice, aliquoted and stored at -20°C. Avoid additional freeze-thaw cycles.

ADDITIONAL REQUIRED MATERIAL

PRODUCT	CATALOG #
mTeSR™1	05850/05870/05875/05857
TeSR™2	05860/05880
DMEM/F12	36254
BD Matrigel™ hESC-qualified matrix	BD Biosciences #354277
Gentle Cell Dissociation Reagent	07174
D-PBS without Ca++ and Mg++	37350
D-PBS, 10X Concentrate, without Ca++ and Mg++	37354
Y-27632 ROCK Inhibitor	07171/07172

DIRECTIONS FOR USE

1.0 PASSAGING CELLS FOR DEFINITIVE ENDODERM INDUCTION

Note: BD Matrigel™-coated plates should be prepared in advance and be brought to room temperature (15 - 25°C) for at least 30 min prior to use. For complete instructions on coating plates with BD Matrigel™ 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 and TeSR™2 (Document #29106) available on our website at www.stemcell.com or contact us to request a copy.

Note: mTeSR™1 or TeSR™2 cultures of human ES and iPS cells are ready for passage when cultures are approximately 70% confluent. Expected cell yields are approximately 10^6 cells per well from a 6-well plate; or 1 - 2 x 10^7 cells from a 10 cm dish.

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. On **Day 0**, warm (37°C) sufficient volumes of mTeSR™1 or TeSR™2, DMEM/F12, and Gentle Cell Dissociation Reagent for passaging. Prepare single-cell passaging medium by adding Y-27632 ROCK inhibitor to mTeSR™1 or TeSR™2 to reach a final concentration of 10 µM.
2. Wash well to be passaged with 1 mL of Ca^{2+}/Mg^{2+}-free phosphate-buffered saline (PBS).
3. Aspirate wash medium and add 1 mL of Gentle Cell Dissociation Reagent.
4. Incubate at 37°C for 8 - 10 min.
5. Dislodge cells by pipetting up and down 1 - 3 times using a pipette with a p1000 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/F12. Wash well once with 1 mL of DMEM/F12 to collect any remaining cells and transfer to the tube, and centrifuge at 300 x *g* for 5 min.
7. Resuspend cells in 1 mL of single-cell passaging medium and count the number of live cells using a hematocytometer.
8. Plate cells at a density of 2.1 x 10^5 per cm^2 (i.e. 2 x 10^6 cells per well) onto BD Matrigel™-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.

2.0 DIFFERENTIATING MONOLAYER CULTURES TO DEFINITIVE ENDODERM

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. On **Day 1**, warm (37°C) sufficient volumes of DMEM/F12 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).

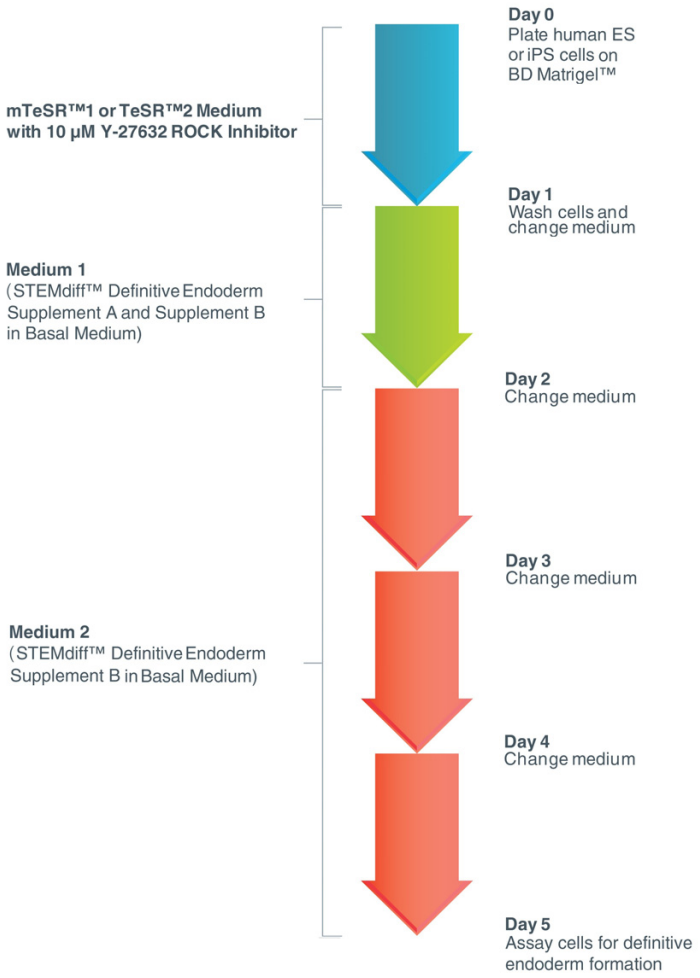
   Note: Supplements should be thawed on ice and kept cold until added to STEMdiff™ Definitive Endoderm Basal Medium.
3. Aspirate medium and wash with 1 mL DMEM/F12.
4. Aspirate wash medium and replace with 2 mL of Medium 1.
5. Incubate at 37°C for 24 hours.
6. On **Day 2**, prepare Medium 2 by diluting 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). Keep 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/F12 is not required at this step or during subsequent media 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). Keep 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 media 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.

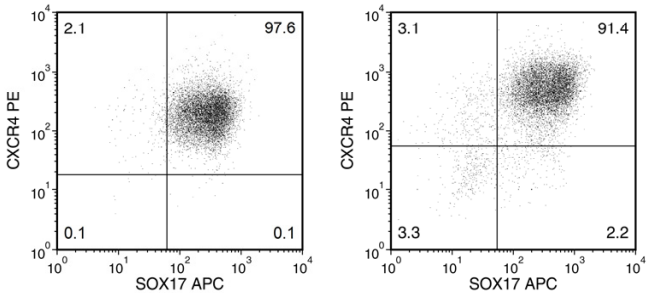
    Note: Differentiation efficiency of some cell lines may peak by Day 4.

SCHEMATIC OF STEMDIFF™ DEFINITIVE ENDODERM KIT PROCEDURE



ASSESSMENT OF DEFINITIVE ENDODERM CELLS

After using the STEMdiff™ Definitive Endoderm Kit, definitive endoderm cells can be measured by flow cytometry. Human ES and iPS cell lines differentiated for 4 - 5 days will typically yield $CXCR4^{+}SOX17^{+}$ cells at 60 - 99% purity. Results may vary depending on cell line used.



Left: H9 human ES cell line. **Right:** WLS-4D1 human iPS cell line. Isotype controls were used to set quadrant gates.

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