

# STEMdiff™ Pancreatic Progenitor Kit



**Serum-free, defined medium for the differentiation of human ES and iPS cells to pancreatic progenitor cells**

Catalog #05120 1 Kit

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## Product Description

STEMdiff™ Pancreatic Progenitor Kit is a serum-free, defined medium system for efficient and reproducible generation of pancreatic progenitor cells from human embryonic stem (ES) and induced pluripotent stem (iPS) cells through four stages of differentiation: 1) definitive endoderm, 2) primitive gut tube, 3) posterior foregut endoderm, and 4) pancreatic progenitor cells. Differentiated cells express key markers of pancreatic progenitor cells, including PDX-1, NKX6.1 and SOX9. The resulting cells can be transplanted into animal models or further matured towards insulin-producing cells using published protocols for the study of developmental biology of insulin-producing beta cells or disease modeling of diabetes. The purity of pancreatic progenitor cells (PDX1<sup>+</sup>NKX6.1<sup>+</sup>) obtained with the STEMdiff™ Pancreatic Progenitor Kit is typically in the range of 66.5 - 74.5%.

STEMdiff™ Pancreatic Progenitor Kit has been optimized for differentiation of cells maintained in mTeSR™1.

## Product Information

The following components are sold as a complete kit (Catalog #05120) and are not available for individual sale.

PRODUCT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
STEMdiff™ Pancreatic Stage 1 Basal Medium	05121	75 mL	Store at -20°C.	Stable for 12 months from date of manufacture (MFG) on label.
STEMdiff™ Pancreatic Stage 2 - 4 Basal Medium	05122	265 mL	Store at 2 - 8°C.	Stable for 12 months from date of manufacture (MFG) on label.
STEMdiff™ Pancreatic Supplement 1A	05123	240 µL	Store at -20°C.	Stable for 12 months from date of manufacture (MFG) on label.
STEMdiff™ Pancreatic Supplement 1B	05124	720 µL	Store at -20°C.	Stable for 12 months from date of manufacture (MFG) on label.
STEMdiff™ Pancreatic Supplement 2A	05125	240 µL	Store at -20°C.	Stable for 12 months from date of manufacture (MFG) on label.
STEMdiff™ Pancreatic Supplement 2B*	05126	720 µL	Store at -20°C.	Stable for 12 months from date of manufacture (MFG) on label.
STEMdiff™ Pancreatic Supplement 3*	05127	720 µL	Store at -20°C.	Stable for 12 months from date of manufacture (MFG) on label.
STEMdiff™ Pancreatic Supplement 4*	05128	1200 µL	Store at -20°C.	Stable for 12 months from date of manufacture (MFG) on label.

\*This product 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.

## Materials Required But Not Included

PRODUCT NAME	CATALOG #
mTeSR™1	05850
Corning® Matrigel® hESC-qualified matrix	Corning 354277
Vitronectin XF™ kit	07190
DMEM/F-12 with 15 mM HEPES	36254
Gentle Cell Dissociation Reagent	07174
Y-27632	72302
D-PBS (Without Ca <sup>++</sup> and Mg <sup>++</sup> )	37350

## Directions for Use

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

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 #29106) available on our website at [www.stemcell.com](http://www.stemcell.com), or contact us to request a copy. Matrix-coated plates should be prepared in advance and brought to room temperature (15 - 25°C) for at least 30 minutes prior to use.

### A) PASSAGING CELLS FOR DIFFERENTIATION TO PANCREATIC PROGENITOR CELLS

For optimal product performance, passage human ES or iPS cells using the protocol as outlined in this section, before proceeding with Differentiation of Human ES/iPS Cells to Pancreatic Progenitor Cells (section B).

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

This protocol is specific to human ES and iPS cells cultured in mTeSR™1 medium. The following instructions are for use with 6-well plates in a monolayer differentiation culture set up. Indicated volumes are for a single well. If using alternative cultureware, adjust volumes accordingly.

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 D-PBS (Without Ca<sup>++</sup> and Mg<sup>++</sup>).
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 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/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  $2.6 \times 10^5$  cells/cm<sup>2</sup> (i.e.  $2.5 \times 10^6$  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. Proceed to section B.

### B) DIFFERENTIATION OF HUMAN ES/iPS CELLS TO PANCREATIC PROGENITOR CELLS

This section is divided into two parts: Preparation of Media followed by Differentiation Protocol.

#### PREPARATION OF MEDIA

There are 6 medium formulations required for the 4 stages of the differentiation protocol. Prepare each medium as required in the Differentiation Protocol. Medium 1A and Medium 1B may both be prepared on Day 1 of Stage 1; Medium 2A and Medium 2B may both be prepared on Day 1 of Stage 2. Store all media at 2 - 8°C until warmed (37°C) for use.

NOTE: After preparing the required volumes of media, remaining supplement may be aliquoted and stored at -20°C. Do not exceed the shelf life of the supplement. After thawing aliquoted supplements, use immediately. Do not re-freeze.

#### **Medium 1A (Stage 1 Day 1)**

Prepare the volume of Medium 1A (Stage 1 Basal Medium + Supplement 1A + Supplement 1B) required for Day 1 of Stage 1 (2 mL/well). The following example is for preparing 2 mL of Medium 1A. For other volumes, adjust accordingly.

1. Thaw entire bottle of Stage 1 Basal Medium 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 the aliquots, use immediately or store at 2 - 8°C for up to 2 weeks. Do not re-freeze.
2. On Day 1 of Stage 1, warm (37°C) 2 mL of Stage 1 Basal Medium.
3. Thaw Supplement 1A and Supplement 1B on ice.
4. Add 20 µL of Supplement 1A and 20 µL of Supplement 1B to 1.96 mL of Stage 1 Basal Medium. Mix well. Use immediately.

**Medium 1B (Stage 1 Day 2)**

Prepare the volume of Medium 1B (Stage 1 Basal Medium + Supplement 1B) required for Day 2 of Stage 1 (2 mL/well). The following example is for preparing 2 mL of Medium 1B. For other volumes, adjust accordingly.

1. Add 20 µL of cold (2 - 8°C) Supplement 1B to 1.98 mL of cold (2 - 8°C) Stage 1 Basal Medium. Mix well. Store at 2 - 8°C.

**Medium 2A (Stage 2 Day 1)**

Prepare the volume of Medium 2A (Stage 2 - 4 Basal Medium + Supplement 2A + Supplement 2B) required for Day 1 of Stage 2 (2 mL/well). The following example is for preparing 2 mL of Medium 2A. For other volumes, adjust accordingly.

1. On Day 1 of Stage 2, warm (37°C) 2 mL of Stage 2 - 4 Basal Medium.
2. Thaw Supplement 2A and Supplement 2B on ice.
3. Add 20 µL of Supplement 2A and 20 µL of Supplement 2B to 1.96 mL of Stage 2 - 4 Basal Medium. Mix well. Use immediately.

**Medium 2B (Stage 2 Day 2)**

Prepare the volume of Medium 2B (Stage 2 - 4 Basal Medium + Supplement 2B) required for Days 2 and 3 of Stage 2 (total of 4 mL/well). The following example is for preparing 4 mL of Medium 2B. For other volumes, adjust accordingly.

1. Add 40 µL of cold (2 - 8°C) Supplement 2B to 3.96 mL of cold (2 - 8°C) Stage 2 - 4 Basal Medium. Mix well. Store at 2 - 8°C.

**Medium 3 (Stage 3)**

Prepare the volume of Medium 3 (Stage 2 - 4 Basal Medium + Supplement 3) required for Days 1, 2, and 3 of Stage 3 (total of 6 mL/well). The following example is for preparing 6 mL of Medium 3. For other volumes, adjust accordingly.

1. On Day 1 of Stage 3, thaw Supplement 3 on ice.
2. Add 60 µL of Supplement 3 to 5.94 mL of cold (2 - 8°C) Stage 2 - 4 Basal Medium. Mix well. Store at 2 - 8°C.

**Medium 4 (Stage 4)**Stage 4 Day 1

Prepare the volume of Medium 4 (Stage 2 - 4 Basal Medium + Supplement 4) required for Days 1, 2, and 3 of Stage 4 (total of 6 mL/well). The following example is for preparing 6 mL of Medium 4. For other volumes, adjust accordingly.

1. On Day 1 of Stage 4, thaw Supplement 4 on ice.
2. Add 60 µL of Supplement 4 to 5.94 mL of cold (2 - 8°C) Stage 2 - 4 Basal Medium. Mix well. Store at 2 - 8°C.
3. From the remaining Supplement 4, aliquot the volume required for Day 4 of Stage 4 and store at -20°C.

NOTE: If there is still remaining Supplement 4, it may be aliquoted and stored at -20°C for use in future experiments. Do not exceed the shelf life of the supplement.

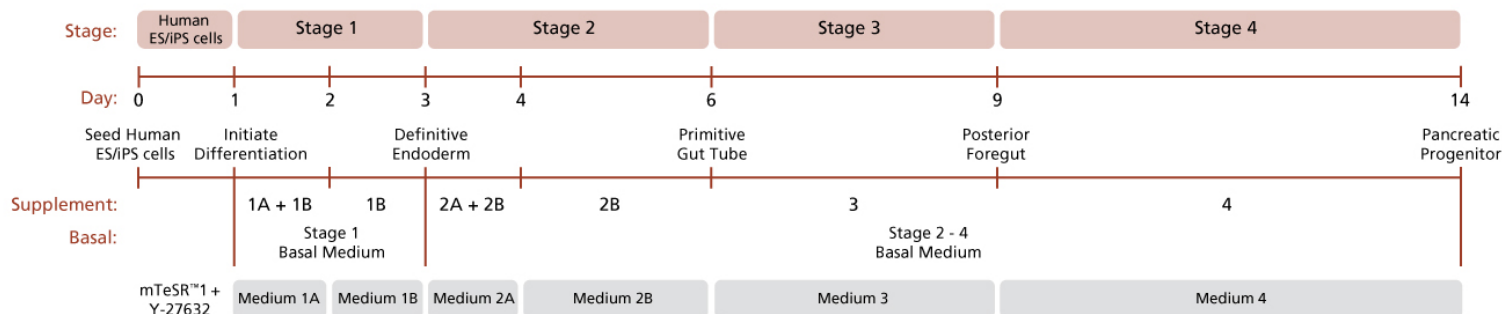
Stage 4 Day 4

Prepare the volume of Medium 4 (Stage 2 - 4 Basal Medium + Supplement 4) required for Days 4 and 5 of Stage 4 (total of 4 mL/well). The following example is for preparing 4 mL of Medium 4. For other volumes, adjust accordingly.

1. On Day 4 of Stage 4, thaw the aliquoted Supplement 4 on ice.
2. Add 40 µL of cold (2 - 8°C) Supplement 4 to 3.96 mL of cold (2 - 8°C) Stage 2 - 4 Basal Medium. Mix well. Store at 2 - 8°C.

## DIFFERENTIATION PROTOCOL

## Schematic for the Generation of Pancreatic Progenitor Cells from Human ES/iPS Cells Using STEMdiff™ Pancreatic Progenitor Kit\*



\*Perform daily medium changes as described in the protocol below.

The following instructions are for use with 6-well plates. Indicated volumes are for a single well. If using alternative cultureware, adjust volumes accordingly.

**Stage 1 (2 days):**

- On Day 1 of Stage 1, prepare Medium 1A and Medium 1B (see Preparation of Media). Store Medium 1B at 2 - 8°C until required.
- Aspirate medium from wells and replace with 2 mL of warm (37°C) Medium 1A per well.
- Incubate at 37°C for 24 hours.
- On Day 2 of Stage 1, warm (37°C) Medium 1B.
- Aspirate medium from wells and add 2 mL of Medium 1B per well.
- Incubate at 37°C 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 (3 days):**

- On Day 1 of Stage 2, prepare Medium 2A and Medium 2B (see Preparation of Media). Store Medium 2B at 2 - 8°C until required.
- Aspirate medium from wells and replace with 2 mL of warm (37°C) Medium 2A per well.
- Incubate at 37°C for 24 hours.
- On Day 2 of Stage 2, warm (37°C) only the volume of Medium 2B required for Day 2 of Stage 2 use (i.e. 2 mL per well). Store remaining Medium 2B at 2 - 8°C.
- Aspirate medium from wells and add 2 mL of Medium 2B per well.
- Incubate at 37°C for 24 hours.
- On Day 3 of Stage 2, warm (37°C) the remaining Medium 2B (i.e. 2 mL per well).
- Aspirate medium from wells and add 2 mL of Medium 2B per well.
- Incubate at 37°C for 24 hours. Proceed to Stage 3.

**Stage 3 (3 days):**

- On Day 1 of Stage 3, prepare Medium 3 (see Preparation of Media).
- Warm (37°C) only the volume of Medium 3 required for Day 1 of Stage 3 use (i.e. 2 mL per well). Store remaining Medium 3 at 2 - 8°C.
- Aspirate medium from wells and add 2 mL of Medium 3 per well.
- Incubate at 37°C for 24 hours.
- On Day 2 of Stage 3, warm (37°C) only the volume of Medium 3 required for Day 2 of Stage 3 use (i.e. 2 mL per well). Store remaining Medium 3 at 2 - 8°C.
- Aspirate medium from wells and add 2 mL of Medium 3 per well.
- Incubate at 37°C for 24 hours.
- On Day 3 of Stage 3, warm (37°C) the remaining Medium 3 (i.e. 2 mL per well).

24. Aspirate medium from wells and add 2 mL of Medium 3 per well.
25. Incubate at 37°C for 24 hours. Proceed to Stage 4.

**Stage 4 (6 days):**

26. On Day 1 of Stage 4, prepare Medium 4 (see Preparation of Media).
27. Warm (37°C) only the volume of Medium 4 required for Day 1 of Stage 4 use (i.e. 2 mL per well). Store remaining Medium 4 at 2 - 8°C.
28. Aspirate medium from wells and add 2 mL of Medium 4 per well.
29. Incubate at 37°C for 24 hours.
30. On Day 2 of Stage 4, warm (37°C) only the volume of Medium 4 required for Day 2 of Stage 4 use (i.e. 2 mL per well). Store remaining Medium 4 at 2 - 8°C.
31. Aspirate medium from wells and add 2 mL of Medium 4 per well.
32. Incubate at 37°C for 24 hours.
33. On Day 3 of Stage 4, warm (37°C) the remaining Medium 4 (i.e. 2 mL per well).
34. Aspirate medium from wells and add 2 mL of Medium 4 per well.
35. Incubate at 37°C for 24 hours.
36. On Day 4 of Stage 4, prepare Medium 4 (see Preparation of Media). Prepare only the volume required for Days 4 and 5 of Stage 4 (i.e. 4 mL per well).
37. Warm (37°C) only the volume of Medium 4 required for Day 4 of Stage 4 use (i.e. 2 mL per well). Store remaining Medium 4 at 2 - 8°C.
38. Aspirate medium from wells and add 2 mL of Medium 4 per well.
39. Incubate at 37°C for 24 hours.
40. On Day 5 of Stage 4, warm (37°C) the remaining Medium 4 (i.e. 2 mL per well).
41. Aspirate medium from wells and add 2 mL of Medium 4 per well.
42. Incubate at 37°C for 24 hours.
43. On Day 6 of Stage 4, cells are ready to be assayed for the formation of pancreatic precursor cells or carried forward into more specialized assays including transplantation into animal models.

## 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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