STEMdiff™ Pancreatic Progenitor Kit

Serum-free 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 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+NKX6.1+) obtained with 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 (Catalog #85850) or mTeSR™ Plus (Catalog #100-0276).

Product Information

The following components are sold as a complete kit (Catalog #05120) 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™ Pancreatic Stage 2 - 4 Basal Medium	05122	265 mL	Store at 2 - 8°C.	Stable for 24 months from date of manufacture (MFG) on label.
STEMdiff™ Definitive Endoderm Supplement MR (100X)	05112	350 µL	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™ Pancreatic Supplement 2A	05125	240 µL	Store at -20°C.	Stable for 24 months from date of manufacture (MFG) on label.
STEMdiff™ Pancreatic Supplement 2B*	05126	720 µL	Store at -20°C.	Stable for 24 months from date of manufacture (MFG) on label.
STEMdiff™ Pancreatic Supplement 3*	05127	720 µL	Store at -20°C.	Stable for 24 months from date of manufacture (MFG) on label.
STEMdiff™ Pancreatic Supplement 4*	05128	1200 µL	Store at -20°C.	Stable for 24 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 #
Corning® Matrigel® hESC-Qualified Matrix	Corning 354277
Costar® 6-Well Flat-Bottom Plate, Tissue Culture-Treated	38015
DMEM/F-12 with 15 mM HEPES	36254
D-PBS (Without Ca++ and Mg++)	37350
Gentle Cell Dissociation Reagent	07174
Hausser Scientific™ Bright-Line Hemocytometer	100-1181
mTeSR™1	85850
Y-27632	72302

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® 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 (Document #10000005505) or mTeSR™Plus (Document #10000007757), available at 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 (section B).

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

This protocol is specific to human ES and iPS cells cultured in mTeSR™1 medium. If using mTeSR™ Plus, we recommend reducing the seeding density by 50% in combination with a 24 hour transition from mTeSR™ Plus to mTeSR™1 before initiation of pancreatic progenitor differentiation (i.e. seed in mTeSR™1 on Day 0, then proceed with Stage 1 Day 1).

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

Day 0

1. Coat tissue culture-treated plate(s) with Corning® Matrigel® at 37°C for at least 2 hours prior to cell seeding to minimize the risk of detachment of monolayers during differentiation. Follow dilution instructions as indicated by manufacturer.
2. 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 a final concentration of 10 µM.
3. Wash the well to be passaged with 1 mL of D-PBS (Without Ca++ and Mg++).
4. Aspirate wash medium and add 1 mL of Gentle Cell Dissociation Reagent.
5. Incubate at 37°C for 6 - 10 minutes. Gently tap plates to see if cells are starting to detach from plate.
6. 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.
7. 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.

   NOTE: Avoid over trituration or leaving cells for extended time in DMEM/F-12 to maintain high cell viability.
8. Resuspend cells in 1 mL of Single-Cell Passaging Medium and count the number of live cells using a hemocytometer.
9. Aspirate diluted Matrigel® from coated plates (prepared in step 1) and immediately add viable cells at a density of 2.6 x 10^5 cells/cm^2 (i.e. 2.5 x 10^6 cells/well). Cells should be approximately 90 - 100% confluent on Day 1.

   NOTE: Differentiation efficiencies are sensitive to cell seeding density. If cells are not 90 - 100% confluent on Day 1, do not extend culture time before starting differentiation; restart the experiment with adjusted seeding cell number. Optimal seeding cell density can vary depending on the growth characteristics of the cell line used.
10. Incubate at 37°C for 24 hours.
11. Proceed to section B for differentiation.

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 supplements may be aliquoted and stored at -20°C. Do not exceed the shelf life of the supplements. After thawing aliquoted supplements, use immediately. Do not re-freeze.

Medium 1A (Stage 1 Day 1)

Prepare the volume of Medium 1A (Endoderm Basal Medium + Supplement MR + Supplement CJ) 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 Endoderm Basal Medium.
3. Thaw Supplement MR and Supplement CJ on ice.
4. Add 20 µL of Supplement MR and 20 µL of Supplement CJ to 1.96 mL of Endoderm Basal Medium. Mix well. Use immediately.

Medium 1B (Stage 1 Day 2)

Prepare the volume of Medium 1B (Endoderm Basal Medium + Supplement CJ) 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 CJ to 1.98 mL of cold (2 - 8°C) Endoderm 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.

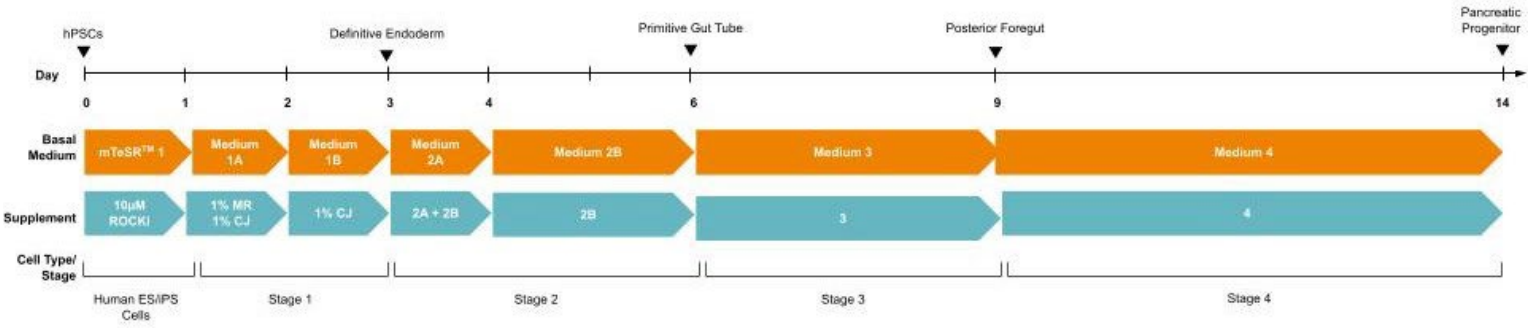
Stage 4 Day 4

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.

NOTE: Avoid disrupting the monolayer when aspirating and adding medium to wells. Slowly add medium alongside the culture vessel wall and avoid aspirating the cells or scratching the monolayer when removing medium. A small volume of medium can remain in the well without affecting differentiation.

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):

1. 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.
2. Aspirate medium from wells and replace with 2 mL of warm (37°C) Medium 1A per well.
3. Incubate at 37°C for 24 hours.
4. On **Day 2 of Stage 1**, warm (37°C) Medium 1B.
5. Aspirate medium from wells and add 2 mL of Medium 1B per well.
6. 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):

7. 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.
8. Aspirate medium from wells and replace with 2 mL of warm (37°C) Medium 2A per well.
9. Incubate at 37°C for 24 hours.
10. 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.
11. Aspirate medium from wells and add 2 mL of Medium 2B per well.
12. Incubate at 37°C for 24 hours.
13. On **Day 3 of Stage 2**, warm (37°C) the remaining Medium 2B (i.e. 2 mL per well).
14. Aspirate medium from wells and add 2 mL of Medium 2B per well.
15. Incubate at 37°C for 24 hours. Proceed to Stage 3.

Stage 3 (3 days):

16. On **Day 1 of Stage 3**, prepare Medium 3 (see Preparation of Media).
17. 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.
18. Aspirate medium from wells and add 2 mL of Medium 3 per well.
19. Incubate at 37°C for 24 hours.
20. 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.
21. Aspirate medium from wells and add 2 mL of Medium 3 per well.
22. Incubate at 37°C for 24 hours.
23. 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.

Assessment of Pancreatic Progenitor Cells and Endocrine Differentiation

For guidance on pancreatic progenitor evaluation and differentiation into insulin-producing islet clusters, refer to Zhao et al.

Related Products

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

References

Zhao J et al. (2023) Differentiation of human pluripotent stem cells into insulin-producing islet clusters. J Vis Exp 196 doi: 10.3791/64840.