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JAX_DPC: Protocol for definitive endoderm induction of human induced pluripotent stem cells

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We use this protocol and it's working

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Abstract

The definitive endoderm forms as the bottom layer of trilaminar embryonic disc and develops the innermost germ layer of the embryo. It is the progenitor of the epithelial lining that further differentiates into organs of both the digestive tract and respiratory system. Studying endoderm models offers opportunity to investigate early embryonic development, cell fate mapping, and lineage analysis. This monolayer protocol is adapted from the STEMdiff™ Definitive Endoderm Kit and describes a method for induction of definitive endoderm from human induced pluripotent stem cells (hiPSCs).



Materials

MATERIALS

mTeSR plus (STEMCELL technologies)

KnockOut Serum Replacement (ThermoFisher Scientific, 10828028)

KnockOut DMEM (Thermo
Fisher Scientific, 10829018)

Neurobasal Medium (Gibco, 21103049)

L-glutamine (Gibco, 25030081)

MEM non-essential amino acids (NEAA) (Gibco),

penicillin-streptomycin (Gibco),

b-mercaptoethanol (Gibco, 21985023)

N2 supplement (100X) (ThermoFisher, 17502048)

B27 supplement without vitamin A 50X (ThermoFisher, 12587010)

D-(+)-Glucose solution (Sigma, G8644)

Glutamax (100x) (Gibco, 35050-061)

HEPES pH7.4 (Fisher, 15630080)

BDNF (R&D Systems, 248-BDB-010)

GDNF (R&D Systems, 212-GD-010)

Accutase (StemCell Technologies, 7922)

Reagents and Consumables:

- 1 STEMdiff™ Definitive Endoderm Kit (STEMCELL Technologies, #05110)
 - STEMdiff™ Endoderm Basal Medium (STEMCELL Technologies, #05111)
 - STEMdiff™ Definitive Endoderm Supplement MR (100X) (STEMCELL Technologies, #05112)
 - STEMdiff™ Definitive Endoderm Supplement CJ (20X) (STEMCELL Technologies, #05113)
- mTeSR™ Plus (STEMCELL Technologies, #100-0276)
Corning® Matrigel® Basement Membrane Matrix, LDEV-free (Corning, 354234)
DMEM/F-12 with 15 mM HEPES (STEMCELL Technologies, #36254)
Gibco™ DPBS, no calcium, no magnesium (ThermoFisher, 14190144)
Gentle Cell Dissociation Reagent (STEMCELL Technologies, #100-0485)
Y-27632 (dihydrochloride) (STEMCELL Technologies, #72304)
Gibco™ Trypan Blue 0.4% (ThermoFisher, 15250061)
Corning® Costar® TC-Treated 6-Well Plates (Millipore-Sigma, CLS3516)

Procedure:

- 2 Maintain iPSCs in a Matrigel coated 6-well plate with 2 mL of mTeSR Plus media. Perform daily media change until the well is approximately 70-80% confluent for Day 0.
- 3 **Day 0**
 - 3.1 Coat 6-well plate in Matrigel
 - a. Resuspend a 100 uL aliquot of Matrigel in 6 mL of DMEM/F-12 and add 1 mL to each well of a 6-well plate.
 - b. Place plate in a 37°C incubator for at least 2 hours
 - 3.2 Warm sufficient volumes of mTeSR plus and DMEM/F-12 to 37°C
 - 3.3 Prepare plating media by adding Y-27632 to mTeSR Plus for a final concentration of 10 µM
 - a. Add 2 uL of 5 mM Y-27632 for every 1 mL of mTeSR
 - 3.4 Taking the plate of iPSCs, wash the well with 1 mL of D-PBS
 - 3.5 Aspirate the wash and add 1 mL of Gentle Cell Dissociation Reagent

- 3.6 Incubate plate at 37°C for 10 minutes
- 3.7 While cells are incubating
 - a. Remove the Matrigel coated plate (from Step 1) from the incubator. Aspirate the Matrigel and wash each well with 1 mL of DMEM/F-12
 - b. Add 2 mL of plating medium (mTeSR + Y-27632)
 - c. Place plate back into incubator until ready to seed cells
- 3.8 After 10 minutes, triturate cells to create a single cell suspension and transfer the cells to a 15 mL conical tube containing 1 mL of mTeSR. Rinse well with an additional 1 mL of mTeSR and transfer to the same conical tube
- 3.9 Centrifuge cells at 300 x g for 5 minutes
- 3.10 Carefully aspirate out supernatant and resuspend cell pellet in 1 mL of plating medium (mTeSR + Y-27632).
- 3.11 Count cells by mixing 10 uL of cell suspension with 10 uL of Trypan Blue and count using hemocytometer
- 3.12 Plate cells at a density of 1×10^6 cells per well to the prepared plate (Step 7).
 - a. Cells should be 90-100% confluent on Day 1
- 3.13 Incubate cells at 37°C for 24 Hours

4 **Day 1**

- 4.1 Warm sufficient volumes of DMEM/F-12 and STEMdiff™ Definitive Endoderm Basal Medium for Day 1 use
- 4.2 Prepare **Medium 1** as follows:
 - a. Thaw STEMdiff™ Definitive Endoderm Supplement MR and STEMdiff™ Definitive Endoderm Supplement CJ on ice
 - b. Dilute both supplements 1 in 100 in STEMdiff™ Endoderm Basal Medium
- 4.3 Aspirate medium from the well and wash with 1 mL of DMEM/F-12



4.4 Aspirate DMEM/F-12 and replace with 2 mL of Medium 1

4.5 Incubate at 37°C for 24 hours

5 Day 2

5.1 Prepare **Medium 2**. Prepare sufficient Medium 2 to be used on Days 2, 3, and 4

- Thaw STEMdiff™ Definitive Endoderm Supplement CJ on ice
- Add Supplement CJ to cold (2 - 8°C) STEMdiff™ Endoderm Basal Medium at a 1 in 100 dilution
- Store media for days 3 and 4 media at 2 - 8°C

5.2 Warm sufficient volume of Medium 2 necessary for Day 2 (2 mL per well) to 37°C

5.3 Taking the plate from the incubator, aspirate medium and add 2 mL of Medium 2

5.4 Incubate at 37°C for 24 hours

6 Day 3

6.1 Warm sufficient volume of Medium 2 necessary for Day 3 (2 mL per well) to 37°C

6.2 Taking the plate from the incubator, aspirate medium and add 2 mL of Medium 2

6.3 Incubate at 37°C for 24 hours

7 Day 4

7.1 Warm sufficient volume of Medium 2 necessary for Day 4 (2 mL per well) to 37°C



7.2 Taking the plate from the incubator, aspirate medium and add 2 mL of Medium 2

7.3 Incubate at 37°C for 24 hours

8 **Day 5**

8.1 Cells are ready to be assayed for the formation of definitive endoderm