

Apr 14, 2025

JAX_DPC: Protocol for mesoderm induction of human induced pluripotent stem cells

 Forked from [JAX_DPC: Protocol to produce human dorsal forebrain organoids](#)

DOI

<https://dx.doi.org/10.17504/protocols.io.bp2l68bmzgqe/v1>

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Protocol Citation: Cole Lorig, Juliana Alcoforado Diniz, Paul Robson 2025. JAX_DPC: Protocol for mesoderm induction of human induced pluripotent stem cells. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.bp2l68bmzgqe/v1>



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Protocol status: Working

We use this protocol and it's working

Created: April 09, 2025

Last Modified: April 14, 2025

Protocol Integer ID: 126472

Keywords: pluripotent stem cells the mesoderm, early mesoderm cell, differentiating human pluripotent stem cell, human pluripotent stem cell, pluripotent stem cell, induced pluripotent stem cell, stemdiff™ mesoderm induction medium, protocol for stemcell, cell fate mapping, early embryonic development, investigation of early embryonic development, middle layer of the trilaminar embryonic disc, protocol for mesoderm induction, stemcell, early mesoderm model, mesoderm induction, studying early mesoderm model, trilaminar embryonic disc, mesoderm, cell, cell type, lineage analysis, internal reproductive organ

Funders Acknowledgements:

JAX MorPhiC DPC

Grant ID: 1 UM1 HG012651-01

Abstract

The mesoderm, the middle layer of the trilaminar embryonic disc formed during gastrulation, gives rise to a wide range of cell types that form the musculoskeletal, circulatory, and lymphatic systems as well as organs such as kidney and internal reproductive organs. Studying early mesoderm models allows for investigation of early embryonic development, cell fate mapping, and lineage analysis. This monolayer protocol, adapted from the protocol for STEMCELL Technologies™ STEMdiff™ Mesoderm Induction Medium, describes a method for differentiating human pluripotent stem cells (hPSCs) to early mesoderm cells.



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 mTeSRTM Plus (STEMCELL Technologies, #100-0276)
STEMdiffTM Mesoderm Induction Medium (STEMCELL Technologies, #05220)
Corning[®] Matrigel[®] Basement Membrane Matrix, LDEV-free (Corning, 354234)
DMEM/F-12 with 15 mM HEPES (STEMCELL Technologies, #36254)
GibcoTM DPBS, no calcium, no magnesium (ThermoFisher, 14190144)
Gentle Cell Dissociation Reagent (STEMCELL Technologies, #100-0485)
Y-27632 (dihydrochloride) (STEMCELL Technologies, #72304)
GibcoTM 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 μ M
 - 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 Remove prepared plate from incubator (Step 7) and plate cells at a density of 5×10^5 cells per well
- 3.13 Incubate cells at 37°C for 24 Hours
- 4 **Day 1-4:** Repeat the following steps each day
 - 4.1 Warm STEMdiff™ Mesoderm Induction Medium to room temperature
 - 4.2 Aspirate medium from well and replace with 3 mL of STEMdiff™ Mesoderm Induction Medium
 - 4.3 Incubate cells at 37°C for 24 Hours
- 5 **Day 5**
 - 5.1 Cells are ready to be assayed for the formation of early mesoderm

