Definitive Endoderm Induction Medium

Stem Cell Media

Cat. # SCM302

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.
NOT FOR HUMAN OR ANIMAL CONSUMPTION.

Pack Size: 50 ml

Store at -20ºC

Data Sheet

Background

The definitive endoderm generates the epithelial lining of the respiratory and digestive tracts as well as cell of the thyroid, thymus, lungs, liver and pancreas. The definitive endoderm induction medium (SCM302) is a defined, animal component-free, ready-to-use serum-free medium for efficient differentiation of human pluripotent stem cells (ES/iPSCs) to definitive endoderm (DE) cells.

The 3-day differentiation protocol is simple and has been validated on multiple human iPS cell lines. Differentiated cells express high levels (>85%) of endoderm markers including CXCR4, c-Kit, Sox-17 and FOXA2 and can be further differentiated into specialized endodermal cell lineages. Definitive Endoderm Induction Medium can be used to generate 3D organoids such as hiPS derived colon (Cat. No. SCC300) and lung organoids.

Storage

Upon receipt, store at -20ºC. When ready to use, thaw overnight at 2-8°C. Once thawed, mix thoroughly and aliquot into smaller volumes (i.e. 10 mL), use immediately and store at 2-8°C for up to 1 week. Do not re-freeze. Unused aliquots may be stored at -20°C until the expiry date.

Quality Control

- Appearance: Clear liquid/no particulates
- Sterility Tested: No growth
- Endotoxin: <2 EU/mL
- pH: 7.0-7.4
- Mycoplasma: Negative
- Functional Assay: >85% CXCR4+ c-Kit+ double positive after 3 days of induction.

References

- Hay D, et al. (2008) Highly efficient differentiation of hESCs to functional hepatic endoderm requires ActivinA and Wnt3a signaling. *PNAS* 26(105):12301-12306.
- Rezania A, et al. (2012) Maturation of human embryonic stem cell-derived pancreatic progenitors into functional islets capable of treating pre-existing diabetes in mice. *Diabetes* 61:2016-2029.

Representative Images

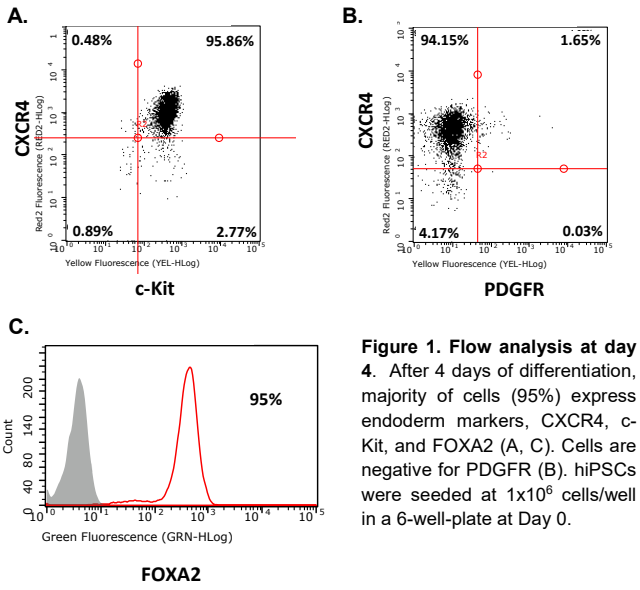

Figure 1. Flow analysis at day 4. After 4 days of differentiation, majority of cells (95%) express endoderm markers, CXCR4, c-Kit, and FOXA2 (A, C). Cells are negative for PDGFR (B). hiPSCs were seeded at $1x10^6$ cells/well in a 6-well-plate at Day 0.

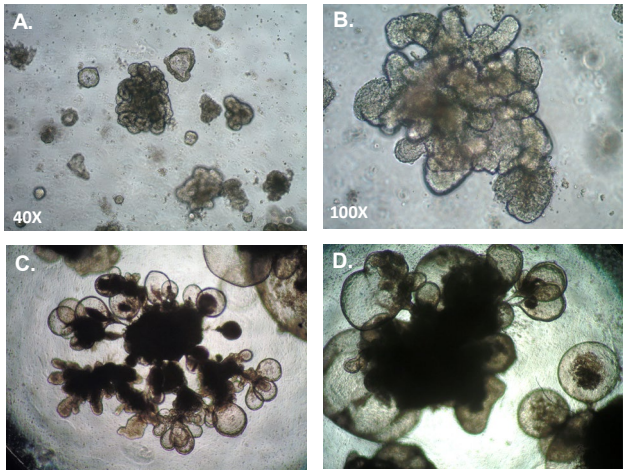

Figure 2. Definitive Endoderm Induction Medium can be used to generate DE cells that can be further differentiated into 3D colon and lung organoids. Human PBMC-iPS derived colon organoids at P4 **(A, B).** Human HFF-iPS derived lung organoids at day 74 (**C**) and day 78 (**D**).

Rev.1.0/2019-05-23/SCM302/NJA

Materials required but not included

Human ES/iPS Expansion Medium:	PluriSTEM Expansion Media (Cat. No. SCM130) or mTeSR™1 (Stem Cell Technologies Cat. No. 85850) may be used
Stem Cell Qualified ECM Gel:	Cat. No. CC130
Y-27632	Cat. No. SCM075
AccuMax™ Cell Detachment Solution	Cat. No. SCR006
DMEM/F12, with HEPES, L-Glutamine	Cat. No. DF-041-B
1X D-PBS (without Ca++ and Mg++)	Cat. No. BSS-1006-B
6-well tissue culture plates	Thermo Fisher Cat. No. 140675

Important Notes Before Starting

- Use high quality undifferentiated human ES/iPS cells. Remove any areas of spontaneous differentiation before starting. Failure to remove differentiated areas may result in decreased efficiency of DE induction. For complete instructions on culture and maintenance of high quality undifferentiated human ES/iPS cells, please refer to the PluriSTEM manual (Cat. No. SCM130) which is available on our website: www.emdmillipore.com/
- Human ES/iPS cells should be cultured on tissue culture-treated plates coated with 1:20 dilution of Stem Cell Qualified ECM Gel (Cat. No. CC131-5ML). For detailed coating instructions, please refer to the Stem Cell Qualified ECM Gel (Cat. No. CC131-5ML) datasheet which is available on our website: www.emdmillipore.com/
- The induction protocol is based on culture of human ES/iPS cells in feeder-free conditions.
- The induction protocol is based on single cells dissociation and require the use of ROCKi, Y-27632 (Cat. No. SCM075; 10 μm final) to enhance cell survival.
- Aliquot the Human DE Induction medium in 10 mL aliquots and store at -20C until ready to use. 10 mL should be sufficient for a 1 well reaction. After thaw, store aliquots at 2-8°C during the duration of the induction period.

Protocol

Day 0: Start with high quality undifferentiated human ES/iPS cells that are ~70-80% confluent and contain <5% differentiated cells. The following protocol is for differentiation of one well of a 6-well tissue culture treated plate. Indicated volumes are for a single well. Adjust volumes as necessary.

1. Prepare Single Cell Passaging Media: Add Y-27632 to 7-10 mL Human ES/iPS Expansion Media to a final concentration of 10 μM.
2. Prepare an ECM Gel coated 6-well plate: Please refer to Cat. No. CC131-5ML datasheet for detailed instructions.
3. Aspirate the medium. Wash the well with 2 mL of DMEM/F12 or 1X PBS. Aspirate and add 1 mL of AccuMax (Cat. No. SCR006) to the well. Incubate for 5-6 minutes at 37°C. Tap the plate firmly against the palm of your hand to help dislodge the cells.
4. Add 1 mL of the Single Cell Passaging Media (from step 1) to the well. Pipet up and down 1-3 times with a 5 mL pipettor to dislodge the cells. Be careful to not introduce any bubbles.
5. Collect the dissociated cells (~2 mL volume) to a 15 mL conical tube. Add 1 mL Single Cell Passaging Media to the well and collect any remaining cells and transfer to the 15 mL conical tube containing the cell suspension. Centrifuge at 800 rpm for 5 minutes. Aspirate.
6. Resuspend the cell pellet in 1 mL of Single Cell Passaging Media. Count the total number of live cells using Trypan blue and a hemocytometer.
7. Add $1x10^6$ cells per well to an ECM Gel coated 6-well plate. The media used should be the Stem Cell Passaging Media (from step 1); Total volume = 3 mL per well. Incubate at 37°C overnight.

Day 1 to Day 3: Definitive endoderm differentiation

1. Aspirate the medium from the well. Add 2 mL of DE Induction Medium to the well and incubate at 37°C overnight.
2. Repeat step 1 for days 2 and days 3.

Day 4: Set up flow analysis for definitive endoderm markers (CXCR4, c-Kit, SOX17, and FOXA2) or continue differentiation towards more specialized lineages.

Please visit www.millipore.com for additional product information, test data and references
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