Hindgut Induction Medium

Stem Cell Media

Cat. # SCM303

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 hindgut is the region of the gut tube lying at the distal third of the transverse colon and gives rise to splenic flexure, descending colon, sigmoid colon and rectum. Additionally, the endodermal layer of the hindgut region forms the epithelial lining of the urinary bladder and urethra. The main function of the hindgut is to absorb most of the water and nutrients remaining from partially digested food.

The Hindgut Induction Medium is a defined ready-to-use serum-free media used for the efficient differentiation of definitive endoderm cells to CDX-2 positive hindgut endoderm cells. The 4-day differentiation protocol is simple and has been validated on two human iPS cell lines. Differentiated cells express high levels of the hindgut endoderm marker, CDX-2. CDX-2 hindgut endoderm cells can be further differentiated into specialized lineages such as intestinal colon organoids after embedding in Matrigel and cultured in 3dGRO™ Human Colon Organoid Expansion Medium (SCM304).

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: >60% CDX-2+ expression after 4 days of differentiation from iPSC derived DE cells.

Representative Images

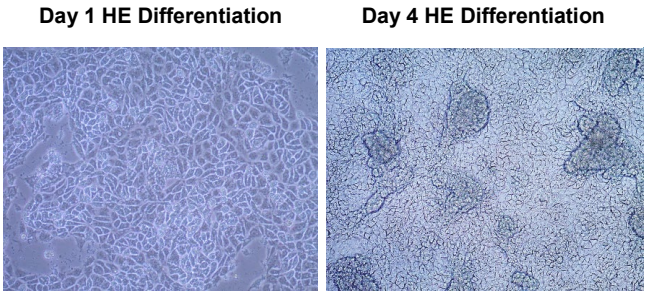

Figure 1. Morphological changes during hindgut endoderm differentiation. Human iPSC derived definitive endodermal cells change morphology over a 4-day differentiation in Hindgut Induction Medium and start forming 3-dimensional CDX-2+ hindgut progenitor cells.

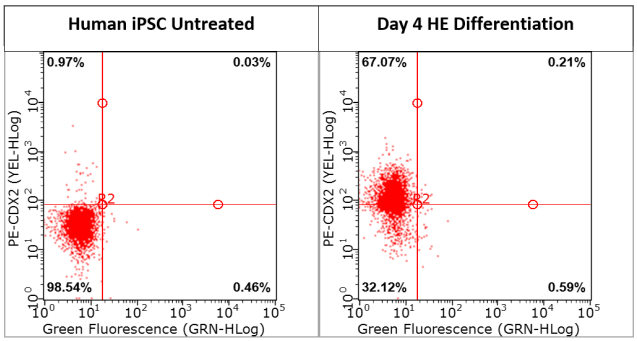

Figure 2. Flow analysis after 4-day hindgut endoderm differentiation. The majority of hindgut endoderm cells should express CDX-2+ after 4 days of differentiation in the Hindgut Induction Medium. In order to form colon organoids ≥60% CDX-2 expression must be reached after 4-days of hindgut endoderm differentiation.

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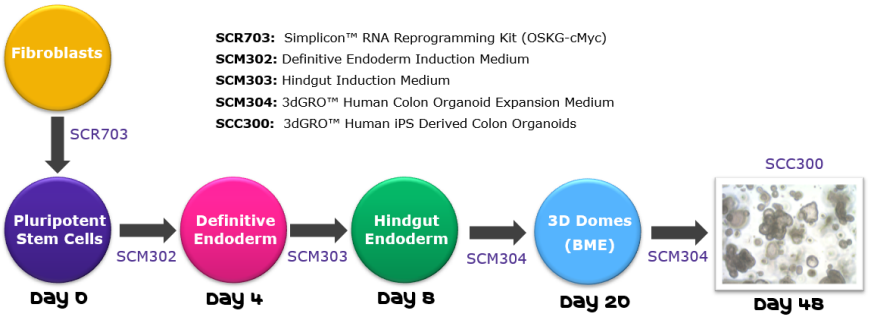
Human Colon Organoid System



Important Notes Before Starting:

- The Hindgut Induction Medium is a continuation of the stepwise process to differentiate human pluripotent ES/iPS cells to definitive endoderm cells to hindgut endoderm and eventually to colon organoids (see above).
- For complete instructions on generating definitive endoderm cells from human pluripotent stem cells, please refer to the datasheet for SCM302, Definitive Endoderm Induction Medium.
- **The hindgut induction media protocol starts from day 4 of definitive endoderm cells.**
- Pluripotent stem cells should be maintained in parallel as they will be used at Day 8 as a negative control for the QC flow analysis.

Protocol (Hindgut Endoderm Differentiation from DE cells)

Day 4: Start with a 6-well plate of cells that have been determined through flow analysis to be >85% positive for definitive endoderm markers (CXCR4, c-Kit, Sox17).

1. Remove a 6-well plate containing day 4 definitive endoderm cultures from the incubator
2. Aspirate the medium from each well to be differentiated.
3. Wash each well with 3 mL 1X PBS (Cat. No. BSS-1006-B)
4. Add 2 mL of Hindgut Induction Medium (Cat. No. SCM303) into each well. Incubate in a 5% CO_2, 37°C incubator overnight.

Day 5 to Day 8:

5. Repeat steps 2-4 every day for a total of 4 days.

Day 8: Quality Control of Hindgut differentiation.

6. Set up flow analysis for CDX-2, a hindgut endoderm marker. Expression of CDX-2 must be $\geq$ 60% to continue with the colon organoid differentiation. PE Mouse Anti-Human CDX-2 may be obtained from Becton Dickinson 563428.

Troubleshooting:

Observation	Possible Cause(s)	Recommended Action
Floating cells observed 24 hours after Hindgut Endoderm (HE) induction.	It is normal to observe many floating cells after 24 hours of HE induction. 10-20% and up to 50% floaters may be observed in some cases. This may depend upon the human iPS cells used. **Note**: Having floating cells during HE induction may generate a purer HE population.	Continue with HE differentiation. Cells will grow back to confluency during the 4 days of differentiation.
>50% floating cells observed after 24 hours HE induction.	Seeding density of human ES/iPS cells at day 0 may be suboptimal. Human ES/iPS cells may not be healthy or contain >5% spontaneously differentiated cells. Cells at day 4 of definitive endoderm (DE) induction may not be confluent. If confluency is too low (60% or less), most cells may die during the DE induction and this in turn may affect the efficiency of HE induction.	Start with high quality undifferentiated human ES/iPS cell that are 70-80% confluent and contain <5% differentiated cells.
Very little floating cells (<10%) after 24 hours of HE induction and throughout the whole 4 days of differentiation.	This may be okay for some human iPS cells. However, this is usually an indication that the HE induction may not have been efficient. At the end of HE induction (i.e. Day 8), the QC flow analysis of CDX-2 expression should be $\geq$ 60%.	Use a brand-new bottle of Hindgut Induction Medium. Do not thaw or warm Hindgut Induction Medium at 37°C before use. Make sure to use high quality human ES/iPS at 80% confluent.