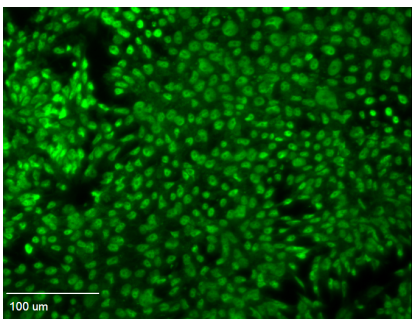
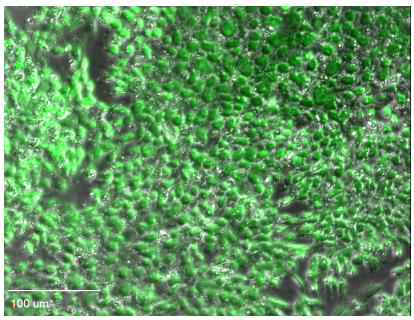
iPS Definitive Endoderm Differentiation Media

CET.DIFF.DEM-250

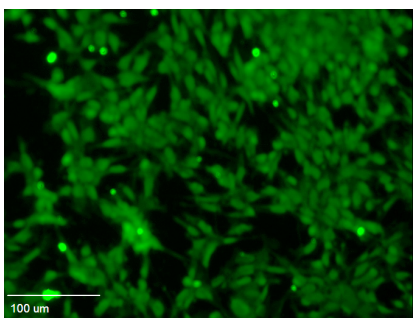
CET's Definitive Endoderm (DE) Differentiation Media is designed to convert human iPSC into definitive endoderm. Unlike competing media, CET's DE Differentiaton Media protocol is a one-step process that is quick, reliable, economical and consistent. By following this protocol, greater than 97% of plated iPSC convert into DE within 4 days. Conversion into DE allows the investigator to create cells from the liver, thymus, pancreas, thyroid, lung and intestines.

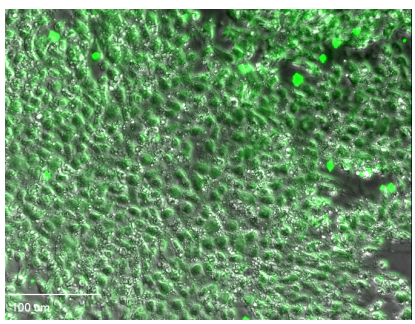
SOX17





FOXA2





Note: Once complete media has been formulated, it should be stored at 4°C. Shelf life of complete media is 3 weeks. Avoid extended exposure of the medium to room temperature or higher temperatures. Medium should be equilibrated at room temperature before addition to any cell culture.

Coating Protocol (Day -1):

1. A day prior to plating your iPSC (both normal and disease specific iPS lines are available separately from CET), tissue culture dishes or plates must be coated with a thin layer of Matrigel. Although there are various vendors of ECM matrix, CET's recommends Corning Matrigel that is qualified for use with IPSC cells. Coat dishes or plates according to manufacturer's directions. Those can be found at: https://www.corning.com/media/worldwide/cls/documents/CLS-DL-CC-026%20DL.pdf

iPSC Culture (Day 0-2):

Passage actively growing iPS colonies (with limited differentiation of less than 5%) onto Matrigel coated plates. **Never plate frozen and thawed IPS cells directly onto Matrigel coated surfaces.**

1. To passage iPSC, wash iPSC with pre-warmed 1X Dulbecco's Phosphate Buffered Saline (DPBS). Gently aspirate and add room temperature 1X Versene. Leave tissue culture plate or dish in a laminar flow hood at room temperature. It is not necessary to reintroduce the dish into a 37°C incubator.

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2. At the end of 10 minutes, gently aspirate the Versene. Some cells will dislodge and be aspirated; this is normal. Add CET's iPS growth media without allowing the well to dry. Gently triturate the cells using a 5 mL serological pipette to create cell clumps. Cell clumps should consist of approximately 100-200 cells. Do not be too vigorous in this process since single cells will not replate. **OPTIONAL**: During initial passage of cells onto the Matrigel coated surface, 5 micromolar Y-27632 can be added to CET's iPS Growth Media. If this is done, it must be withdrawn after the first 24 hours. **CET strongly recommends against single cell passaging using Dispase or Accutase since these tend to damage cells and greatly affects their replating efficiency.**

3. Let IPS clumps attach in a 37°C incubator, without disturbing, for 24 hours. Feed with fresh CET iPS Growth Media at the end of 24 hours. If you used Y-27632 during passaging, make sure this media does not contain this Rho Kinase inhibitor.

4. Depending on the iPS line that you are growing, they may have different proliferation or growth rates. However, it is critical to achieve 85% to 90% confluency on a given plate surface before proceeding to the differentiation process.

Differentiation into Definitive Endoderm (Day 2-6):

1. Reconstitute complete CET's Definitive Endoderm Differentiation Media by thawing CET's DE Supplement overnight at 4°C (in a refrigerator). **Never thaw supplement by placing it into a 37oC waterbath.** Add the entire contents of the supplement (10 milliliters) to CET's Definitive Endoderm Base Media. Mix gently. Label, date and place complete DE Differentiation Media in a 4°C refrigerator. CET's Complete Definitive Endoderm Differentiation Media has a shelf life of 3-weeks if stored at 4°C.

2. Pre-warm CET's Definitive Endoderm Differentiation Media to room temperature. **Do not place it into a 37°C waterbath.** Only aliquot the amount necessary to conduct a complete media replacement. CET recommends 200 microliters for a single well of a 96-well plate and 2 milliliters for a single well of a 6-well plate.

3. Gently aspirate the iPSC growth media from the tissue culture dish and introduce CET's Definitive Endoderm Differentiation Media. Place tissue culture dish back into the 37°C incubator.

4. At the end of 48 hours, re-feed the cells with CET's Definitive Endoderm Differentiation Media (See Steps 2-3 above).

5. At the end of 4 days, iPSC will have been converted into Definitive Endoderm and are ready for downstream applications.

Confirmation of Definitive Endoderm:

1. At the end of 4 days, cells can be screened via immunofluorescence for the expression of FOXA2 and SOX-17.

All of CET's iPS cell lines are foot-print free, feeder-free and virus-free. All procedures must be done by trained personnel with experience in cell culture. All procedures must be done in a sterile fashion using a laminar flow hood and all cells must be cultured in a 37°C incubator with 95% relative humidity and 5% CO_2. Although it is not necessary, better growth and differentiation are seen in tri-gas incubators capable of reducing Oxygen concentration to 5%.

Table 1. Preparation of 250 ml complete Induced Pluripotent Stem Cell Definitive Endoderm Media

Brand	Amount for 250 mL	Product	Catalog Number
CET	240 mL	CET DE Media	CET.DIFF.DEM-250
CET	10 mL	CET Growth Supplement	CET.DIFF.DEM-250
Any	2.5 mL	Antibiotic/ Antimycotic solution	Refer to Manufacturer's Catalog Number

Certificate of Analysis

All hematopoietic, mesenchymal and multipotent stem cells are evaluated by flow cytometry for specific stem cell mark-ers. All other cells are evaluated either by staining, method of isolation or traditional molecular biology techniques. Data is available upon request.

All growth and differentiation media are evaluated by conducting assays to make sure cells either grow or differentiate as indicated on the media label. Data is available upon request.

All cells are tested for HIV-1, HIV-2, Hepatitis B and Hepatitis C using sensitive PCR based assays. All cells test nega-tive for these viruses. However, all human cells must be used in accordance with established laboratory safety procedures and only under the supervision of trained personnel.

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