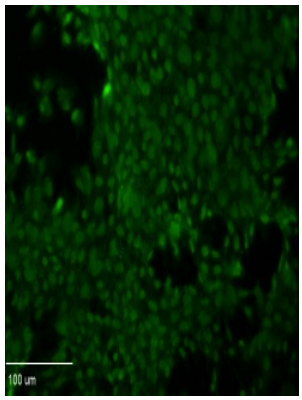
Human Adipose Mesenchymal IPS Cells

CET.IPS.FFAD-500 Store cells at -80°C or in liquid nitrogen



Thawing and Plating Protocol:

This Adipose MSC IPS cell line was reprogrammed from a normal human adipose-derived mesenchymal stem cell. The IPS cell line was reprogrammed using CET's Episomal Reprogramming Mix containing a proprietary mix of vectors (Oct-4, Sox-2, p53 antisense and EBNA-1). IPS cells are fastidious, require regular maintenance and should only be grown by users who have extensive tissue culture experience. Instructions should be followed carefully or cells risk differentiation, slow growth or cell death. All CET IPS cells are subject to a material transfer agreement. Please refer to that document for further details.

Note: Once complete media has been formulated, it should be stored at 4°C. Shelf life of complete media is 14 days. 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.

Thawing Cells:

1. Before thawing the cells, substrate coated dishes should be prepared accordingly. 30 minutes before thawing the IPS cells, the plates must be completely replaced with CET's Complete IPS Growth Media (Table 1) and equilibrated to room temperature.
2. Remove the vial of Adipose MSC IPS cells from liquid nitrogen or -80°C freezer. Thaw the cryovial by immersing the vial in a 37°C water bath without submerging the cap. Swirl the vial gently. The vial is considered thawed when 80% of the contents are liquid and there is a small ice pellet remaining.
3. Spray the outside of the vial with reagent grade alcohol and introduce into a laminar flow hood.

Plating Cells:

4. Introduce the contents of the cryovial into 10 mL of CET's Complete IPS Growth Media in a 15 mL conical. Invert once, gently to mix.
5. Centrifuge for 5 minutes at 200 x g to pellet the IPS cells. Ideally this should be done in a swing bucket rotor. At the end of 5 minutes, a loose pellet should form. Be careful not to shake the tube or bump it against any surface as the cells will re disperse into the solution.
6. Aspirate the supernatant slowly, leaving 200 µL behind. This will contain the IPS cells.
7. Resuspend the IPS cells in an additional 6 mL of pre-warmed CET Complete IPS Growth Media using a serological pipette. Do this gently to avoid shearing the colonies.
8. Gently pipette the resuspended cells on previously coated dishes. One vial of IPS cells contains enough colonies for 6 wells of a standard 6 well tissue culture plate or 3-100 mm tissue culture dishes. Distribute the colonies evenly and gently rock the plate back and forth. The cells should attach over a period of 24 hours.
9. Observe cells microscopically and place the dish in an incubator at 37°C, 5% CO2 and 95% humidity.
10. At the end of 24 hours, aspirate media, but do so gently to avoid shearing the colonies. Replace with fresh, pre-warmed CET Complete IPS Growth media. CET recommends using 10 mL per 100 mm tissue culture dish and 2 mL per well of a 6 well tissue culture plate.
11. Repeat Step 10 every 24 hours. IPS cells will be ready to pass between 5-7 days.

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Table 1. Preparation of 500 ml complete Induced Pluripotent Stem Cell Growth Media

Brand	Amount for 500 mL	Product	Catalog Number
CET	488 mL	CET Base Media	CET.IPS.GMK-500
CET	7 mL	CET Growth Supplement	CET.IPS.GMK-500
Any	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. IPS cells are validated through alkaline phosphatase staining, expression of SSEA-4, TRA 1-60, Nanog and Oct-4 and differentiation studies. 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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