Designation: **iPSC-FFNPC-01**
Induced Pluripotent Stem Cells reprogrammed from episomal cells

CLS order number: Cryovial: 300620-01
500,000 cells/vial

Origin and General Characteristics	
Organism:	Human (Homo sapiens)
Tissue:	episomal tissue
Cell type:	Adipose Mesenchymal Stem Cell
Growth Properties:	Adherent, clonal
Description:	The iPSC-FFNPC-01 cell line was generated by reprogramming normal human adipose-derived mesenchymal stem cells with the Episomal Reprogramming Mix containing several vectors (Oct-4, Sox-2, p53 antisense and EBNA-1 (manufactured by CET, USA; CLS order no.: 850941).
MTA:	All iPS cells are subject to a Material transfer agreement (MTA), please refer to that document for further details.
Culture Conditions and Handling	
Culture Medium:	iPS growth medium (Cat.no. 860200) which comes as a kit comprising the Base medium plus supplement. To prepare 500 ml complete iPS cell growth medium, add 7 ml of the growth supplement to 488 ml of the Base media. Add 5 ml of Antibiotic / Antimycotic solution according to Manufacturer's recommendations. The formulated media should be stored at 4°C for a maximum of 14 days. Before adding to cells, allow the media to equilibrate to room temperature; Still avoid extended exposure to room or higher temperatures.
First-step Medium:	The presence of the ROCK-inhibitor (Y27632, Stem Cell technologies) is necessary for each step the iPS cells are plated or subcultured. Once the cells are counted, they must be resuspended in iPS Growth medium containing Y27632 at concentrations recommended by the supplier. 24 hours later, the ROCK-inhibitor must be removed by adding iPS Growth medium without the ROCK-inhibitor as a supplement.
Tissue culture vessels:	Tissue culture vessels must be precoated using either Matrigel or Synthemax. Please follow recommendations given by the supplier.
Thawing and Plating Protocol:	**Thawing iPS cells**: 1. 30 Minutes before thawing iPS cells, substrate coated dishes must be prepared by completely replacing the plates with iPS growth medium containing the ROCK inhibitor. 2. Remove the vial of iPS-FFHFF cells from liquid nitrogen and gently thaw the vial by immersing it into a water bath prewarmed to 37°C. Take care not to submerge the cap. If a small ice pellet is remaining, consider cells as thawed. 3. Gently spray the vial with reagent grade alcohol for desinfection and then transfer it into a flow hood. **Plating Cells**: 4. Transfer contents of the cryovial into a 15 mL conical tube filled with 10 mL of the iPS Growth medium containing ROCK inhibitor. 5. Centrifuge for 5 minutes at 200 x g, ideally, in a swing bucket rotor centrifuge. A cell pellet should have formed. Gently transfer the conical tube containing the iPS cells to a laminar flow hood. Take care not to loosen the cell pellet. 6. Carefully aspirate the supernatant. Do not aspirate the cell pellet. Leave about 200 µL liquid in the conical tube. iPS cells will be contained in the media. 7. Gently resuspend the cell pellet with 6 mL of pre-warmed iPS Growth medium using a serological pipette. 8. Gently pipette the re-suspended cells and distribute evenly on 3 wells of the 6 well tissue culture plate coated before. Rock the plate back and forth to easily dispense the

	cells. Place the dish in an incubator at 37°C, 5% CO_2 and 95% humidity and allow cells to attach for 24 hours. 10. After 24 hours, gently aspirate media without shearing the colonies. Then replace media with fresh and complete pre-warmed iPS Growth Medium. 2 mL of media per well of a 6 well tissue culture plate is recommended. If other growth surfaces are to be used, add 1 ml media per 1 cm^2 of growth surface. 11. Replace media everyday as described in step 10. iPS cells can be passed within between 5-7 days.
Fluid Renewal:	Daily
Freeze Medium:	CM-ACF (CLS order number 800650, 50ml; 800625, 25 ml)
Freezing recovery:	Slow
Sterility:	Fluorescence (DAPI) test: negative; Mycoplasma specific PCR: negative; Bacteria specific PCR: negative
Biosafety Level:	1
Safety precautions:	If the cryovial is planned to be stored in liquid nitrogen and to be thawed in the future, special safety precautions should be followed: Protective gloves and clothing should be used and a facemask or safety goggles must be worn when transferring frozen samples into or removing from the liquid nitrogen tank. The removal of a cryovial from liquid nitrogen may result in the explosion of the frozen vial creating flying fragments. Caputo, J.L. Biosafety procedures in cell culture. J. Tissue Cult. Methods 11:223-227, 1988. ATCC Quality Control Methods for Cell Lines, 2nd edition, 1992.
Special Features of the Cell Line	
Viruses:	Free of human pathogenic viruses HIV-1, HIV-2, Hepatitis B and Hepatitis C
Validation of pluripotency:	SSEA-4, TRA 1-60, Nanog, Oct-4, alkaline phosphatase live stain
Related Cell Lines:	IPS-FFHFF; IPS-FFAD; IPS-FFMP; IPS-FFAM; IPS-FFCD34; IPS-FFGAU; IPS-FFCYSFIB; IPS-FFCYST; IPS-FFALZ; IPS-FFNPC-01; IPS-FFNPC-02

Certificate of Analysis:	The Certificate of Analysis for each batch can be requested by e-mail at service@clsgmbh.de.

All products are for research use only. Not for diagnostic or therapeutic use. These iPS products are designed and tested to function with the recommended products only. All of the iPS cells are optimized to grow and differentiate in media as recommended above. Although investigators are welcome to formulate their own media, any warranty of sufficient functions cannot be given. Moreover, such third party use will void the manufacturer's obligation to replace cells, should they not function as indicated.

Manufactured by: Cellular Engineering Technologies (CET) Inc., USA

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Recommendations for handling of adherent cell cultures following delivery	
Cryopreserved cells	The cells come deep-frozen shipped on dry ice. Please make sure that the vial is still frozen. If immediate culturing is not intended, the cryovial(s) must be stored below -150°C after arrival. Please follow instructions given above for handling of the IPS cells.

Warranty:	CLS warrants for a high cell viability and culture performance only if the product(s) is (are) stored and cultured according to the information described above. Using cell culture media and supplements other than the ones recommended in this product information may result in satisfactory proliferation and viabilities. CLS, however, does not warrant for cell recovery, proliferation and function if differing formulations are employed.
Disclaimer:	The customer shall not be entitled to employ this product for purposes other than research. Commercial utilization shall not be permitted; in particular, the cell line, its components or materials made therefrom shall not be sold or transferred to any third party. In addition, the term ‘Commercial use’ shall mean any activity by a party for consideration and may include, but is not limited to, use of the product or its components in manufacturing, for providing services, e.g. fee for service testing, in quality control or assurance processes within the manufacturing of products for sale, for therapeutic, diagnostic or prophylactic purposes, or for resale.