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Product Information Sheet

Human iPSC Episomal DNA Reprogramming Mix

Catalog Number: MR1004

Product Overview	
Product Name	**Human iPSC Episomal DNA Reprogramming Mix**
Catalog #	MR1004
Quantity	iPSC Episomal Reprogramming Mix (supports five (5) reactions)
Product Form	One vial of frozen human episomal DNA
Cell Species	Human

Product Description

Proprietary mix of non-integrating episomal DNA, small molecule inhibitors, and glycolysis activators to assist cell reprogramming and maintain cell pluripotency. Reprogramming vectors are based on a pCEP-4 episomal backbone containing a bacterial origin of replication and resistance genes for ampicillin/hygromycin.

The vial contains enough episomal DNA for 5 separate electroporation or transfection steps.

Shipped on dry ice.

Note: This product has been tested using Cellular Engineering Technologies Inc. ("CET") Human iPSC Complete Reprogramming Kit (CAT: MR1003-K) (not included). Although investigators are welcome to use this product with other cell culture media and supplement products, CET cannot and will not guarantee this product's performance. Additionally, using third-party cell culture media and supplements with this product will void CET's warranty should they not function as indicated. Please refer to CET's Terms & Conditions, available at www.cet.bio.

Product Image

iPSC Episomal Reprogramming Mix Preparation	
Defrosting / Preparation	Defrost the iPSC Episomal Reprogramming Mix at 4°C twenty-four hours before Day 0 (see iPSC Reprogramming Protocol below) electroporation or transfection step. Defrost 2.5mL of antibiotic/antimycotic solution (not included) in a 37°C water bath until ice in the tubes is no longer visible. Never defrost the iPSC Episomal Reprogramming Mix using a 37°C water bath. Gently mix by inversion. Immediately disinfect the tubes and the bottle containing the iPSC Episomal Reprogramming Mix with 70% isopropanol (not included).

Recommended Inputs and Ancillary Materials for iPSC Reprogramming

Recommended Inputs *(not included)*

- Target cells to be reprogrammed. CET recommends actively growing fibroblasts of low passage number or a nucleated fraction of whole blood.
- Electroporator and Associated Equipment. This protocol is optimized for the Neon Electroporation System sold by Thermo Fisher. You will have to optimize electroporation parameters for your electroporator, which maximizes cell transfection while minimizing cell death.
- Thermo Fisher Lipofectamine™ 3000. Although an electroporator is strongly recommended, especially for suspension cells, Lipofectamine 3000 may be used for adherent cells amenable to transfection. Please refer to the manufacturer's directions and optimize for your applications.
- Penicillin/Streptomycin/Amphotericin B solution or Penicillin/Streptomycin solution, 100x[1] ("antibiotic/antimycotic solution")
- 70% isopropanol

Adherent Cells

- Thermo Fisher Geltrex™ hESC-Qualifed, Ready-To-Use, Reduced Growth Factor Basement Membrane Matrix
- Dulbecco's Phosphate Buffered Saline (DPBS)
- 0.25% Trypsin-EDTA

Suspension Cells

- Corning Matrigel Matrix

iPSC Reprogramming / iPSC Growth Media Formulation Instructions

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Defrosting / Preparation	*For complete iPSC Reprogramming Media:* Defrost the iPSC Reprogramming Supplement at 4°C twenty-four hours before the reprogramming media is to be prepared and 2.5mL of antibiotic/antimycotic solution (not included) in a 37°C water bath until ice in the tubes is no longer visible. Never defrost the iPSC Reprogramming Supplement using a 37°C water bath. Immediately disinfect the tubes and the bottle containing the iPSC Reprogramming Supplement with 70% isopropanol (not included). *For complete iPSC Growth Media:* Defrost iPSC Growth Supplement at 4°C twenty-four hours before the media is to be prepared and 5mL of antibiotic/antimycotic solution (not included) in a 37°C water bath until ice in the tubes is no longer visible. Never defrost the iPS Growth Supplement using a 37°C water bath. It is normal for iPS Growth Supplement to appear hazy or have suspended solutes. Gently mix by inversion. Immediately disinfect the tubes and the bottle containing the iPS Growth Base Media with 70% isopropanol (not included).
Mixing	*For complete iPSC Reprogramming Media:* Working in a laminar flow hood, remove 2.5 mL of the iPSC Reprogramming Base Media from the bottle and discard it. This and all other procedures must be done in a sterile manner. Add the complete contents of the iPSC Reprogramming Supplement to the iPSC Reprogramming Base Media. Add 2.5mL of the antibiotic/antimycotic solution to the iPS Reprogramming Base Media[1]. Cap the bottle containing the mixed liquid solution and gently swirl it a few times. This formulated media is now considered complete and ready to use with primary cells. *For complete iPSC Growth Media:* Working in a laminar flow hood, remove 12 mL of the iPSC Growth Base Media from the bottle and discard it. This and all other procedures must be done in a sterile manner. Add the complete contents of the iPSC Growth Supplement to the iPSC Growth Base Media. Add 5mL of the antibiotic/antimycotic solution to the iPSC Growth Base Media. Cap the bottle containing the mixed liquid solution and gently swirl it a few times. This formulated media is now considered complete and ready to expand target cells.

iPSC Reprogramming Protocol	
Day -2	**Adherent Cells** • Examine target cells under a microscope. If using fibroblasts, ensure cells are actively growing in the logarithmic phase and are approximately 80% confluent. Do not use cells that are over-confluent or slow growing. • To transfect each target cell, coat 2 wells of a 6-well tissue culture dish with Geltrex (not included). **Suspension Cells** • If you use suspension cells, ensure cell density is $1x10^6$ cells/mL. For each suspension cell to be transfected, coat 1 well of a tissue culture dish with Matrigel (not included).
Day 0	**Adherent Cells** • Withdraw media and wash target cells once with Dulbecco's Phosphate Buffered Saline (not included). Add an appropriate amount of 0.25% Trypsin-EDTA (not included). Incubate for 3-4 minutes in the incubator. Add an equal volume of serum-containing growth media (not included) when the cells are no longer adherent. Make sure media contains NO ANTIBIOTICS/ANTIFUNGALS. Count cells and adjust the density of cells to $1x10^6$ cells/mL. Spin cells to pellet at 200 X G for 5 minutes in a swing bucket rotor. Resuspend the cell pellet in 100 microliters of Neon Electroporation Buffer R. Add 6 microliters of CET iPSC Episomal Reprogramming Mix to this tube. This is approximately 10 micrograms of DNA. Mix gently using a micropipette. • Using a Neon electroporation Tip-100, introduce cells and DNA. Using Buffer E2 for the chamber buffer, electroporate cells at 1650 V for 10 milliseconds for 3 cycles. Immediately after electroporation, place cells in your growth media on the coated 6-well dish. This media must contain NO ANTIBIOTICS /ANTIFUNGALS for the first 24 hours. **Suspension Cells** • Count and spin cells to pellet at 200 X G for 5 minutes in a swing bucket rotor. Cell density should be $1x10^6$ cells/mL. Resuspend the cell pellet in 100 microliters of Neon Electroporation Buffer S. Add 6 microliters of CET iPS Episomal Reprogramming Mix to this tube. This represents approximately 10µg of DNA. Mix gently using a micropipette. Using a Neon electroporation Tip-100, introduce cells and DNA. Using Buffer E2 for the chamber buffer, electroporate cells at 1650 V for 10 milliseconds for 3 cycles. Immediately after electroporation, place cells in your growth media on the coated 6-well dish. This media must contain NO ANTIBIOTICS /ANTIFUNGALS for the first 24 hours.
Days 1-3	**Adherent Cells** • At the end of 24 hours, withdraw growth media and conduct a complete replacement with complete IPS Reprogramming Media. At this time, the use of Antibiotics/Antifungals will not affect cell viability and is recommended. Although exogenous gene expression should start within 12 hours, robust gene expression can be detected by RFP fluorescence at the end of 48 hours. Feed cells every 48 hours with complete IPS Reprogramming Media. **Suspension Cells**

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	• It will take approximately 72 hours post-electroporation for suspension cells to settle and adhere. Therefore, it is critical not to aspirate suspension cells while conducting a media change. At the end of 24 hours, gently tilt the plate so cells settle to the bottom. Using a serological pipette, gently withdraw one-half of the volume of the antibiotic/antifungal-free growth media. Replace with a half volume of complete IPS Reprogramming Media. After 3 days, you can conduct a full replacement with complete IPS Reprogramming Media.
Days 3-14	**Adherent / Suspension Cells** • Perform a full replacement of complete IPS Reprogramming Media every 48 hours. Suspension cells should be adherent by this time. All cells should start becoming more cuboidal or epithelial in appearance and start forming small putative IPS colonies by the end of 14 days.
Day 14 and thereafter	**Adherent / Suspension Cells** • Perform a full media replacement with complete IPS Cell Growth Media every 24 hours. Although it is difficult to predict when mature IPS colonies will emerge, this process should take approximately 17 days post-electroporation. Monitor IPS colonies daily. IPS colonies are ready to be passaged when they have sharp, distinct edges. • For directions on continued passaging and colony expansion, please refer to CET's guide on growing and maintaining IPSCs.

Storage and Stability		
	Storage Temperature	**Storage Time**
iPS Cell Episomal Reprogramming Mix	**-20°C**	**6 months**
iPSC Reprogramming Base Media	4°C	3 months
iPSC Reprogramming Supplement	-20°C	Not applicable (use entire contents)
iPSC Growth Base Media	4°C	3 months
iPSC Growth Supplement	-20°C	Not applicable (use entire contents)
complete medias *(see Media Formulation Instructions)*	4°C	Not applicable
Avoid repeated exposure to room temperature and light.		

[1] These solutions should be portioned in 5mL aliquots, stored at -20C, and never frozen/thawed. Although antimycotics are not necessary, CET highly recommends their usage for long-term cell culture. Antibiotics and antimycotics should not be used as an alternative to proper aseptic techniques.

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