

Product Summary

Human Multipotent Induced Pluripotent Stem Cells (iPSCs)

Catalog Number: CR1002-500

Product Overview	
Product Name	Human Multipotent Induced Pluripotent Stem Cells
Catalog #s	CR1002-500
Quantity	1 vial (approx. 500,000 cells)
Product Form	Frozen
Cell Type	Human Induced Pluripotent Stem Cells
Reagents Needed	Penicillin/Streptomycin/Amphotericin B solution or Penicillin/Streptomycin solution, 100x (not included) ¹

Product Description
<p>Multipotent cells expressing lower levels of MHC Class II, isolated from cord blood, were reprogrammed with our patented episomal, virus-free method using a proprietary mix of vectors, excluding <i>I-Myc</i>, <i>c-Myc</i>, and <i>Lin28</i> transcription factors.</p> <p>The cell line was validated for pluripotency based on colony morphology, alkaline phosphatase staining, and expression of SSEA-4. Cells are free of Mycoplasma and exhibit classical iPS colony morphology and growth characteristics.</p> <p>Vial contains approximately 500,000 cells. Shipped with dry ice.</p> <p>Note: This product is designed and tested to function with Cellular Engineering Technologies Inc. ("CET") product MR1001 Human iPS Cell Growth Media (not included). Although investigators are welcome to use this product with other media formulations, CET cannot and will not guarantee this product's performance. Additionally, such use of third-party media 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.</p>



Media Formulation Instructions (for MR1001-K Human iPS Cell Growth Media Kit <u>not</u> included)	
Defrosting the iPS Cell Growth Supplement	<ol style="list-style-type: none"> Defrost the iPS Cell Growth Supplement at 4°C (the day before the media is prepared) and 5 mL of antibiotic/antimycotic solution (not included) in a 37°C water bath until the ice in the tubes is no longer visible. Never defrost the iPS Cell Growth Supplement in a 37°C water bath. It is normal for the iPS Cell Growth Supplement to appear hazy or have suspended solutes. Gently mix by inversion. Immediately disinfect the tubes and the bottle containing the iPS Cell Growth Base Media with 70% isopropanol (not included).
Mixing	<ol style="list-style-type: none"> Working in a laminar flow hood, remove 12mL of iPS Cell Growth Base Media (not included with cells) from the bottle and discard. This and all other procedures must be done in a sterile manner. Add the complete contents of the iPS Cell Growth Supplement to the iPS Cell Growth Base Media. Add 5mL of the antibiotic/antimycotic solution to the iPS Cell Growth Base Media¹. Cap the bottle containing the mixed liquid solution and gently swirl a few times. This formulated media is now considered complete media and ready to use with cells.

Cell Thawing and Plating Instructions	
Cell thawing	<ol style="list-style-type: none"> Before thawing the cells, substrate-coated dishes should be prepared accordingly. Thirty (30) minutes before thawing the iPS cells, the coating solution on the plates must be entirely replaced with complete media (see Media Formulation Instructions) containing five (5) uM ROCK Inhibitor Y-27632 (not included) and equilibrated to room temperature. Remove the Human Multipotent Induced Pluripotent Stem Cells vial from the dry ice or a storage unit.

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	<ol style="list-style-type: none"> 4. Defrost the vial of cells in a 37°C water bath with constant, moderate agitation until ice in the ampoule is barely visible. DO NOT OVERTHAW. 5. Immediately disinfect with 70% isopropanol (not included).
Cell plating	<ol style="list-style-type: none"> 1. Working in a laminar flow hood, open the vial and transfer the contents to a sterile fifteen (15) mL tube. 2. Very slowly, add approximately nine (9) mL of complete media (see Media Formulation Instructions) containing five (5) uM ROCK Inhibitor Y-27632, pre-warmed to 37°C before use. 3. Centrifuge suspended cells at 200 x g for 10 minutes. 4. Decant the medium and gently resuspend the pellet in 6 mL of complete media containing 5 uM ROCK Inhibitor Y-27632. Do this gently to avoid shearing the colonies. 5. Gently pipette the resuspended cells onto the previously coated dishes. One vial of iPSCs contains enough colonies to seed six (6) wells of a standard six (6)-well tissue culture plate or three (3)- one-hundred (100) mm tissue culture dishes. 6. Distribute the colonies evenly and gently rock the plate back and forth. Place the dish in an incubator at 37°C, 5% CO₂, and 95% humidity. 7. After 24 hours, aspirate media from the dish and replenish with fresh complete media (WITHOUT 5uM ROCK Inhibitor Y-27632), pre-warmed to 37°C before use. 8. Repeat media changes every 24 hours.
Observation and expansion	<ul style="list-style-type: none"> - The cells should attach over 24 hours. It is normal for these cells to grow slowly initially for one week after thawing and for some colonies to be shed during media changes. - Subculture cells at a 1:6 split ratio using cell disassociation reagent (not included).

Storage and Stability		
	Storage Temperature	Storage Time
Human Multipotent iPSCs Cat. CR1002-500	Upon arrival, place the cells at a temperature below -130°C, preferably in liquid nitrogen vapor, until ready for use	12 months
Human iPSC Growth Media Kit (not included) Cat. MR1003-K		
iPSC Growth Base Media	4°C	3 months
iPSC Growth Supplement	-20°C	Not applicable (use entire contents)
complete media (see Media Formulation Instructions)	4°C	14 days
<i>Avoid repeated freeze-thaw cycles for cells. Avoid repeated exposure to room temperature and light for media.</i>		

Publications and Product Citations
<p>Engineering toxin-resistant therapeutic stem cells to treat brain tumors Stuckey, DW et al. Stem Cells 2015 FEB Department of Neurology, Massachusetts General Hospital Department of Medical Oncology and Center for Molecular Oncologic Pathology, Dana-Farber Cancer Institute, and Harvard Stem Cell Institute.</p>

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.