iPSCdirect™ Healthy Control Human iPSC Line, SCTi003-A

Catalog #200-0510	1 Vial	1 x 10^7 cells/vial
#100-1028	10 Vials	1 x 10^7 cells/vial

STEMCELL™ TECHNOLOGIES
Scientists Helping Scientists™ | WWW.STEMCELL.COM
TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713
INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM
FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

Product Description

iPSCdirect™ is a high-density, single-use source of singularized induced pluripotent stem cells (iPSCs) that are ready-to-use immediately after thaw. Developed under the same quality management system as Healthy Control Human iPSC Line, Female, SCTi003-A (Catalog #200-0511), each vial of iPSCdirect™ has undergone rigorous quality control procedures and demonstrates high levels of consistency and performance. iPSCdirect™ contains 10 million viable cells per vial which are immediately ready for use in downstream applications, such as differentiation using STEMdiff™ media products.

The highly consistent and robust nature of iPSCdirect™ removes the costly and time-consuming process of developing and quality control (QC) testing master and working cell banks for your iPSC research, as well as the need to keep your iPSCs in long-term culture. Save time and reduce passage-to-passage variability by starting with the same source of highly QC-tested iPSCs for each of your experiments Of note, iPSCdirect™ was derived from an αβ T cell and has undergone VDJ rearrangement.

iPSCdirect™ Healthy Control Human iPSC Line, SCTi003-A, was derived from peripheral blood mononuclear cells (PBMCs) from a 48-year-old female donor. SCTi003-A is karyotypically stable, demonstrates trilineage differentiation potential, expresses markers of the undifferentiated state, and was reprogrammed using a non-integrating reprogramming technology. The cells may be used as a healthy control for a multitude of pluripotent stem cell research applications, including downstream differentiation to lineage-specific cell types such as cardiomyocytes, neural progenitors and hepatocyte-like cells. iPSCdirect™ is manufactured with our leading mTeSR™ medium and is compatible with several STEMdiff™ media products, including STEMdiff™ Ventricular Cardiomyocyte Differentiation Kit and STEMdiff™ SMADi Neural Induction Kit.

Stability and Storage

Cells are frozen in a cryopreservation medium containing dimethyl sulfoxide (DMSO). Product stable at -135°C or colder for 12 months from date of receipt. Short-term storage of cells (< 1 month) at -80°C is acceptable, but should be minimized to ensure maximum stability. Thawed samples must be used immediately.

Precautions

Cell Screening: iPSC master cell banks are screened for AAV2, BK virus, Epstein-Barr Virus, Hepatitis A, Hepatitis B, Hepatitis C, Herpes Simplex 1 and 2, Herpes Virus Type 6, 7, and 8, HIV-1, HIV-2, HPV-16, HPV-18, Human Adenovirus, Human Cytomegalovirus, Human Foamy Virus, Human T-Lymphotropic Virus, John Cunningham Virus, LCMV, Parvovirus B19, Sarbecovirus (SARS Virus), Seoul Virus, Corynebacterium Bovis, and Mycoplasma (Human Comprehensive CLEAR Panel) by PCR. Commercial iPSC banks are tested for the absence of Mycoplasma by PCR. As testing cannot completely guarantee that the donor was virus-free, THIS PRODUCT SHOULD BE TREATED AS POTENTIALLY INFECTIOUS and only used following appropriate handling precautions such as those described in biological safety level 2.

Storage of frozen cell products in the vapor phase of a liquid nitrogen storage tank is recommended. Storage in the liquid phase can result in cross-contamination if the vial breaks or is not sealed properly. Storage in the liquid phase also increases the potential for liquid nitrogen to penetrate the vial and cause it to explode when removed from storage. Use of a face shield is required as a safety precaution when transferring cells from one container to another. When handling this product, do not use sharps such as needles and syringes.

STEMCELL cannot guarantee the biological function or any other properties associated with performance of cells in a researcher's individual assay or culture systems. STEMCELL assures the cells will meet the specifications only when assessed immediately after thawing by our test methods.

FOR IN VITRO RESEARCH USE ONLY. NOT APPROVED FOR DIAGNOSTIC, THERAPEUTIC, OR CLINICAL APPLICATIONS. NOT APPROVED FOR HUMAN OR VETERINARY USE IN VIVO.

Donor Information

Age†	48	
Sex‡	Female	
Diagnosis†	Clinically unaffected at donation	
Ethnicity and/or Race†	White	
Ancestry‡	0% African 78.2% European	0% East Asian 21.8% South Asian
Height‡	168 cm	
Weight‡	62.1 kg	
BMI‡	22.1 kg/m^2	
Blood type‡	B–	
HLA Haplotype‡	HLA Class I A*24:02:01G, 26:01:01G B*07:02:01G, - C*07:02:01G, -	HLA Class II DRB1*15:01:01G, - DRB3*-, - DRB4*-, - DRB5*01:01:01G, - DQB1*06:02:01G, - DPB1*02:01:02G, 04:01:01G
ClinVar Analysis‡§	Five pathogenic or likely pathogenic variants in the following genes: AGXT, KLKB1, NQO1, RUNX1, and SLC12A3.	
Tobacco Use†	Non-smoker	

† Self-declared
‡ Calculated
§ Based on data from the 1000 Genomes Project, the average person has 18 pathogenic or likely pathogenic variants.

Preparation of Reagents and Materials

iPSCdirect™ Healthy Control Human iPSC Line, SCTi003-A, was manufactured using mTeSR™ Plus, and mTeSR™ Plus supplemented with CloneR™2 is the recommended Seeding Medium. Thawed cells should be seeded into tissue culture-treated cultureware pre-coated with Corning® Matrigel® hESC-Qualified Matrix (Corning Catalog #354277) or CellAdhere™ Laminin-521.

For instructions on preparing complete mTeSR™ Plus and coated cultureware, refer to the Technical Manual: Maintenance of Human Pluripotent Stem Cells in mTeSR™ Plus (Document #10000007757), available at www.stemcell.com, or contact us to request a copy.

Seeding Medium

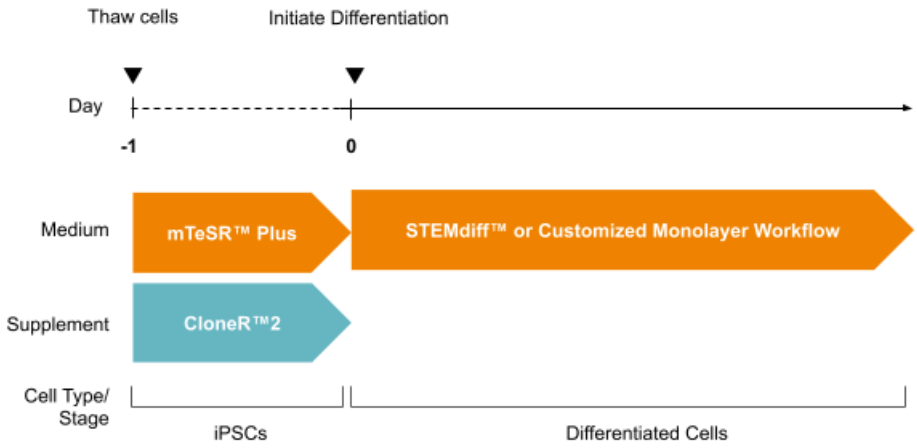
The following example is for preparing 25 mL of Seeding Medium. If preparing other volumes, adjust accordingly. For more information, refer to the Product Information Sheet for CloneR™2 (Document #10000011289), available at www.stemcell.com, or contact us to request a copy.

1. Thaw CloneR™2 at room temperature (15 - 25°C).

   NOTE: If not used immediately, store at 2 - 8°C. Do not exceed the shelf life of the supplement. Alternatively, aliquot and store at -20°C. After thawing the aliquots, use immediately. Do not re-freeze.
2. Prepare complete mTeSR™ Plus.
3. Add 2.5 mL of CloneR™2 to 22.5 mL of complete mTeSR™ Plus. Mix thoroughly by inverting. Do not shake.

   NOTE: If not used immediately, store Seeding Medium at 2 - 8°C for up to 1 week.

Protocol Diagram



Cells are thawed and plated in Seeding Medium (mTeSR™ Plus supplemented with CloneR™2) and incubated overnight according to product instructions. After 24 hours, cells exhibit a healthy monolayer (Figure 1) and are ready for downstream differentiation using STEMdiff™ media or other customized monolayer workflows.

Directions for Use

The following instructions are for seeding cells into coated 6-well plates. If using other cultureware, adjust volumes accordingly. It is important to work quickly in the following steps to ensure high cell viability and recovery.

1. Have all tubes, Seeding Medium, and coated cultureware ready before starting the protocol to ensure that the thawing procedure is completed as quickly as possible. Warm Seeding Medium to room temperature (15 - 25°C).

   NOTE: Do not warm Seeding Medium in a 37°C water bath.
2. Wipe the outside of the vial of cells with 70% ethanol or isopropanol.
3. In a biosafety cabinet, twist the cap a quarter-turn to relieve internal pressure, then retighten.
4. Quickly thaw cells in a 37°C water bath by gently shaking the vial. Remove the vial when a small frozen cell pellet remains. Do not vortex cells.

   NOTE: For optimal thawing in a sterile and controlled manner, use ThawStar® CFT2 Automated Thawing System.
5. Wipe the outside of the vial with 70% ethanol or isopropanol.
6. In a biosafety cabinet, use a 1 mL pipettor to transfer the contents of the cryovials to a 15 mL conical tube containing 5 mL of Seeding Medium.
7. Rinse the vial with 1 mL of Seeding Medium and add it dropwise to the cells while gently swirling the tube.
8. Centrifuge cells at 300 x *g* for 5 minutes at room temperature.
9. Aspirate the medium, leaving the cell pellet intact. Resuspend the cell pellet in 5 mL of Seeding Medium by gently pipetting up and down.
10. Perform a viable cell count.
11. When cells are ready to be plated, aspirate the Corning® Matrigel® or CellAdhere™ Laminin-521 solution from the plate and add Seeding Medium.
12. Plate cells at appropriate densities for desired confluency after 24 hours (see Table 1).

Table 1. Recommended Seeding Density to Achieve Desired Confluency After 24 Hours.[1,2]

LOW CONFLUENCY (0 - 50%)	MEDIUM CONFLUENCY (50 - 80%)	HIGH CONFLUENCY (80 - 100%)
100,000 - 150,000 cells/cm^2	200,000 - 250,000 cells/cm^2	300,000 - 350,000 cells/cm^2

[1] When cultured on recommended matrices: Corning® Matrigel® (Corning Catalog #352477) or CellAdhere™ Laminin-521
[2] Use lower seeding density ranges when using CellAdhere™ Laminin-521 and higher seeding density ranges when using Corning® Matrigel®

13. Place the plates in a 37°C and 5% CO_2 incubator. Move the plate in several quick, short, back-and-forth and side-to-side motions to ensure even distribution of cells. Do not disturb the plate for 24 hours.
14. After 24 hours, observe cultures using a light microscope and ensure the desired confluency has been achieved. Refer to Figure 1 for representative images of culture confluency after 24 hours.

    NOTE: If desired confluency is not achieved within 24 hours, perform a full-medium change with complete mTeSR™ Plus and observe cultures throughout the next 48 hours.
15. When desired confluency is achieved, perform a full-medium change with an appropriate downstream differentiation medium.

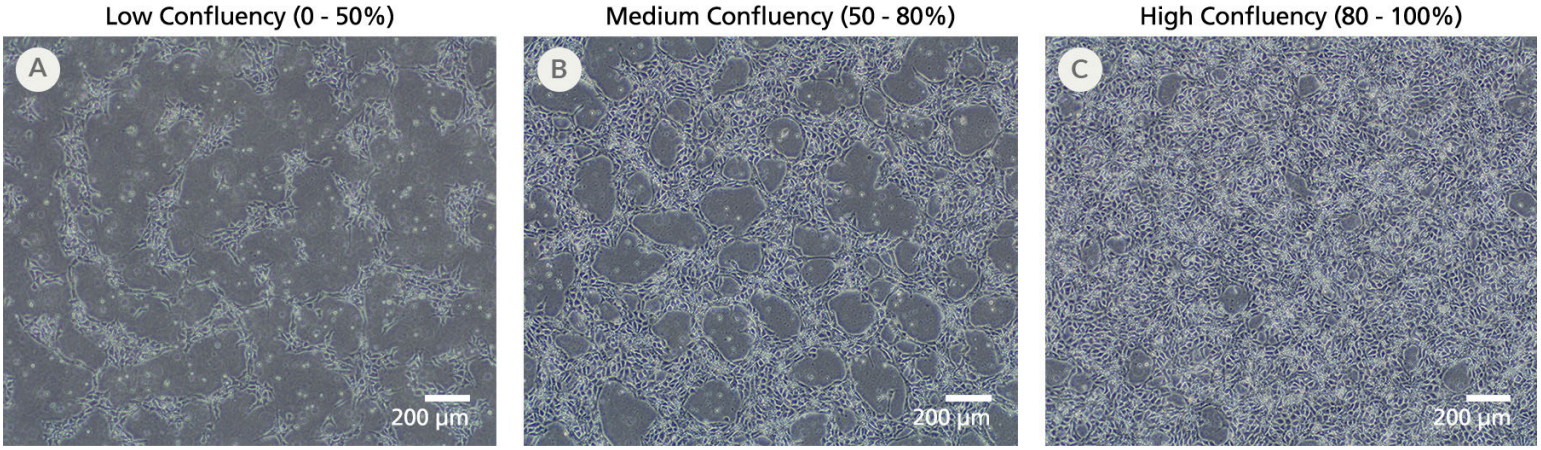


Figure 1. Representative Images of iPSCdirect™ Cells at Low (0 - 50%), Medium (50 - 80%) and High Confluency (80 - 100%) 24 Hours After Plating. iPSCdirect™ cells were recovered in Seeding Medium on Corning® Matrigel® hESC-Qualified Matrix and imaged at 4X magnification.

Accessory Products

PRODUCT NAME	CATALOG #
12-Well Flat-Bottom Plate, Tissue Culture-Treated	38052
96-Well Treated Tissue Culture Plate	27135
Anti-Human OCT4 (OCT3) Antibody, Clone 3A2A20	60093
Anti-Human TRA-1-60 Antibody, Clone TRA-1-60R	60064
CellAdhere™ Laminin-521	200-0117
CellAdhere™ Dilution Buffer	07183
CloneR™2	100-0691
D-PBS (Without Ca++ and Mg++)	37350
Falcon® 6-Well Flat-Bottom Plate, Tissue Culture-Treated	38016
Falcon® 24-Well Flat-Bottom Plate, Tissue Culture-Treated	38021
Falcon® Conical Tubes, 15 mL	38009
Falcon® Serological Pipettes, 2 mL	38002
Human Pluripotent Stem Cell Trilineage Differentiation qPCR Array	07515
mTeSR™ Plus	100-0276
STEMdiff™ SMADi Neural Induction Kit	08581
STEMdiff™ Ventricular Cardiomyocyte Differentiation Kit	05010
ThawStar® CFT2 Automated Thawing System	100-0650