User Guide for

Selleck Human iPSC Enhancer Kit

Cat. No. K2010

Guidelines for Use

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

Selleck Human iPSC Enhancer Kit

Catalog No. K2010

Product Overview

Human induced pluripotent stem cells (human iPSCs), a Nobel prize-worthy discovery, hold enormous potential. They offer great promise for research and clinical applications, such as the modeling of human disease, screening of drug efficacy and safety, and ultimately serve as a source of autologous or allogeneic cells for regenerative medicine. Although rapid advances and adoption of this technology have already been accomplished, human iPSC generation is still a very slow (~4 weeks) and inefficient (≤0.01%) process [1]. The slow kinetics and low efficiency of current human iPSC reprogramming and culture methods have been obstacles to impede their utility in biomedical research and clinical applications.

As future applications of human iPSC technology, genetic alteration for disease correction, loci-specific modulation for reporter systems and clone selection for preferred differentiation potential will require higher throughput and more reliable methods for clone derivation and characterization [2]. Consequently, there is still a tremendous need for a safer, easier and more efficient procedure for human iPSC generation, which would also facilitate identifying and characterizing fundamental mechanisms of reprogramming.

To address some of the challenges of deriving and expanding human iPSCs, Selleck Chemicals launches a novel tool, **Selleck Human iPSC Enhancer Kit**. It is a mixture composed of 4 small molecule inhibitors, including PD0325901, CHIR99021, Thiazovivin, and SB431542, inhibiting **ERK/MEK**, **GSK3**, **Rho/ROCK**, and **TGFβ/ALK** pathway, respectively. The combination of these 4 small molecules has been shown to effectively enhance reprogramming and survival of human iPSCs [2].

Reference:

1. Lin T, et al. A chemical platform for improved induction of human iPSCs. Nat Methods, 2009, 6(11), 805-808.
2. Valamehr B, et al. A novel platform to enable the high-throughput derivation and characterization of feeder-free human iPSCs. Sci Reports, 2012, 2, 213.

Advantages

- Effectively enhances human iPSC reprogramming by 35-fold
- Increases human iPSC yield and viability during recovery from cryopreservation
- Improves recovery and survival of human iPSC after single cell sorting and single cell passage
- Maintains genomic stability and key human iPSC characteristics during long-term single cell culture
- High purity and potency, excellent quality, and competitive price
- Endotoxin, bacteria, and fungi free: ready-to-use as media supplement
- Worry-free: publication-validated

Data

Cat. No.	Inhibitor Name	Concentration 1000×	Targeted Pathway and Function	Information
S1036	PD0325901	5 mM	**ERK/MEK** pathway. To inhibit differentiation.	**MEK**, IC_{50}=0.33 nM.
S1263	CHIR-99021 (CT99021)	1 mM	**GSK3** pathway. To enhance proliferation and viability.	**GSK-3α**, IC_{50}=10 nM; **GSK-3β**, IC_{50}=6.7 nM.
S1459	Thiazovivin	0.4 mM	**Rho/ROCK** pathway. To promote durability.	A drug which dramatically improves the survival of hESCs upon trypsinization. **ROCK**, IC_{50}=0.5 μM.
S1067	SB 431542	2 mM	**TGFβ/ALK5** pathway. To improve reprogramming.	**ALK5**, IC_{50}=94 nM.

These Guidelines for Use provide information intended to aid in the reprogramming, expansion, and recovery from cryopreservation of human iPSCs. Unless otherwise noted, all culture steps should be performed aseptically and in an appropriate biosafety cabinet.

1. Reprogramming

1.1. Materials

1.1.1. Fibroblast medium

- DMEM with L-Glutamine or equivalent
- GlutaMAX or equivalent
- Non-Essential Amino Acids (100 mM)
- Fetal Bovine Serum (FBS) or equivalent

1.1.2. Basic Pluripotent Stem Cell medium

- DMEM/F12 with L-Glutamine or equivalent
- KnockOut Serum Replacement
- 2-Mercaptoethanol or equivalent
- Basic Fibroblast Growth Factor (bFGF)
- Non-Essential Amino Acids
- GlutaMAX or equivalent

1.1.3. Hank's Balanced Salt Solution (HBSS) + Buffer

- HBSS 1X, without calcium, magnesium, and phenol red or equivalent
- Fetal Bovine Serum, Qualified or equivalent
- HEPES (Free Acid) 1M Solution, Liquid
- Penicillin-Streptomycin Solution, 100X or equivalent

1.1.4. Selleck Human iPSC Enhancer Kit (Cat. No. K2010)

1.1.5. Matrigel hESC-Qualified matrix or equivalent

1.1.6. Accutase Cell Detachment solution

1.1.7. 100 mm Cell Culture dishes

1.1.8. 6-well plates

1.1.9. Fetal Bovine Serum, Qualified or equivalent

1.1.10. Human serum albumin (HSA) solution 100 mg/mL or equivalent

1.1.11. Sterile PBS or equivalent

1.1.12. 10 mM Tris, pH 7.6

1.1.13. 0.05% Trypsin (wt/vol)/EDTA or equivalent

1.1.14. Gelatin 0.1% (wt/vol) or equivalent

1.1.15. Polybrene or equivalent

1.1.16. Human fibroblasts (primary or cell line) or other somatic cell type, depending on your process

1.1.17. Reprogramming vector or system (user provided)

1.1.18. Antibodies for Fluorescence Activated Cell Sorting

1.1.19. Antibodies for Imaging

Selleck Human iPSC Enhancer Kit may be used in conjunction with most reprogramming systems. Since many laboratories are using viral vectors to deliver reprogramming genes, these Guidelines for Use are provided with those methods in mind.

1.2. Media Preparation

All media ought to be stored at 4°C, protected from light. To minimize potential degradation of media stocks, we recommend taking appropriate aliquots for daily use. All cell culture media described in this User Guide should pre-warmed to room temperature prior to use, unless otherwise noted.

1.2.1. Fibroblast Medium Preparation (500 mL)

1.2.1.1. To prepare 500 mL Fibroblast medium, combine 50 mL FBS, 5 mL Non-Essential Amino Acids, and 5 mL GlutaMAX.

1.2.1.2. Add DMEM with L-Glutamine (440 mL) up to 500 mL total volume.

1.2.1.3. Fibroblast medium should be stored at 4°C, protected from light. Recommended usage within 2–3 weeks.

1.2.2. Basic FGF Solution

1.2.2.1. Reconstitute a 10 µg vial of Basic Fibroblast Growth Factor in 100 µL of 10 mM Tris, pH 7.6, to yield a 0.1 mg/mL stock solution.

1.2.2.2. Add 100 µL of 0.1 mg/mL stock to 3.9 mL of DMEM/F12 supplemented with 0.1% HSA, yielding a 2.5 µg/mL Basic FGF Solution. This solution can be used fresh or aliquotted and stored at -80°C.

1.2.3. Basic Pluripotent Stem Cell Medium Preparation (500 mL)

1.2.3.1. To prepare 500 mL Basic Pluripotent Stem Cell medium, combine 387.1 mL DMEM/F12 with L-Glutamine, 5 mL GlutaMAX, 5 mL Non-Essential Amino Acids, 0.91 mL 2-Mercaptoethanol, and 100 mL KnockOut Serum Replacement.

1.2.3.2. Add 2 mL Basic FGF Solution to yield a final concentration of 10 ng/mL.

1.2.3.3. Filter the complete medium. This medium should be stored at 4°C, protected from light. Recommended usage within two weeks.

1.2.4. Selleck Human iPSC Enhancer Medium Preparation

1.2.4.1. To prepare Selleck Human iPSC Enhancer medium, add Selleck Human iPSC Enhancer Kit to Basic Pluripotent Stem Cell medium with a ratio of 1:1000 (vol/vol). This medium should be stored at 4°C, protected from light. Recommended usage within two weeks of the preparation of Basic Pluripotent Stem Cell medium.

1.2.5. HBSS+ Buffer (200 mL)

1.2.5.1. To prepare 200 mL HBSS+ Buffer, combine 188 mL HBSS, 8 mL FBS, 2 mL HEPES, and 2 mL Penicillin-Streptomycin in a sterile container. This medium should be stored at 4°C, protected from light. Recommended usage within 2-3 weeks.

1.3. Culture Vessel Preparation

1.3.1. Gelatin-coated plates

1.3.1.1. Before use, warm 0.1% Gelatin Solution to room temperature (15-30ºC).

1.3.1.2. Using aseptic technique, add a sufficient volume of 0.1% Gelatin Solution to cover the bottoms of the wells of your 6-well plates (ie, 2 mL/well).

1.3.1.3. Coat 6-well plates with 0.1% Gelatin solution for at least 30 min at 37°C.

1.3.1.4. Aspirate coating solution immediately prior to plating cells. Rinsing of coating solution in not required.

1.3.2. Matrix-precoated 6-well plates

1.3.2.1. Thaw Matrigel hESC-Qualified matrix on ice. Once thawed, swirl the vial to ensure the matrix is evenly dispersed.

1.3.2.2. Spray the top of the vial with 70% ethanol or equivalent and air dry. Keep product on ice and handle using aseptic technique.

1.3.2.3. Dispense material into appropriate aliquots, using pre-cooled tubes, and refreeze immediately. Avoid

multiple freeze thaws.

1.3.2.4. To prepare plates, add one aliquot of Matrigel hESC-Qualified matrix thawed on ice to 25 mL of cold DMEM/F12 (pre-chilled at 2 to 8 ºC) to coat four 6-well plates (1 mL/well) or three 100 mm dishes (8 mL/dish).

1.3.2.5. Coat plates with matrix for at least 1 hour at room temperature or overnight at 4 ºC.

1.3.2.6. Aspirate coating solution immediately prior to plating cells. Rinsing of coating solution is not required.

Recommended Reprogramming Procedure

Selleck Human iPSC Enhancer Kit (Cat. No. K2010) may be used in conjunction with most reprogramming systems. Since many laboratories are using viral vectors to deliver reprogramming genes to primary fibroblasts, these Guidelines for Use are provided as an example. These methods may be adapted to your cell type and process of choice.

1.4. Transduction of Human Fibroblasts

1.4.1. Culture human fibroblasts in a 100 mm cell culture dish using Fibroblast medium to 50-75% confluency. Wash with Phosphate Buffer Saline (PBS), and then incubate with 3 mL of 0.05% Trypsin/EDTA for 5 min at 37°C.

1.4.2. Add 3 mL of Fibroblast medium to the culture and dissociate cells into a single-cell suspension by gentle trituration.

1.4.3. Transfer to a 15 mL conical tube and centrifuge at 250 x*g* for 5 min at 4°C and aspirate the supernatant.

1.4.4. Re-suspend the cell pellet in Fibroblast medium and determine cell number using a hemacytometer.

1.4.5. Plate 1×10^5 cells per well in a Gelatin-coated 6-well plate and incubated overnight. Ensure that the plates were previously coated with 0.1% gelatin for minimum of 30 min at 37°C.

1.4.6. Following the appropriate safety procedures for your vector system, quickly thaw the appropriate set of viruses needed for the experiment. Ensure that safety precautions are taken when handling live virus solutions, including the bleaching of all materials that come in contact with live virus.

1.4.7. Prepare viruses as you normally would for reprogramming (including vector ratios, in the case of multiple vectors).

1.4.8. Add Fibroblast medium equal to the total virus volume, and supplement with polybrene (for a final concentration of 4 µg/mL).

1.4.9. Add 1 mL of the viral vector mixture to each well, and spin cells in the 6-well plate by centrifugation at 650 x*g*, at 32°C, for 1 hr (spin infection); alternatively, omit the centrifugation step and simply add the viral vector mixture.

1.4.10. Incubate for 7-12 hrs (overnight) in a standard tissue culture incubator at 37°C with 5% CO_2.

1.4.11. Remove and appropriately discard all of the media, and wash the cells with PBS three times to ensure minimal virus carryover to the remaining culture steps.

1.4.12. Add fresh Fibroblast medium and continue to culture at 37°C/5% CO_2.

1.4.13. Continue to Section 1.5.

1.5. Seeding Transduced Cells on matrix with Selleck Human iPSC Enhancer Kit

1.5.1. After culturing the transduced cells for two days (or more depending on your reprogramming system and the confluency of the culture), wash the transduced cells with pre-warmed sterile PBS and incubate them with 1 mL 0.05% Trypsin/EDTA for 3 min at 37°C.

1.5.2. Quench the trypsin activity by adding an equal volume of 50% Fibroblast medium/50% Selleck Human iPSC Enhancer Medium and dissociate cells to single cell suspension using gentle trituration. Centrifuge the mixture at 250 x*g* for 5 min at 4°C.

1.5.3. Aspirate the supernatant and re-suspend the cells in fresh 50% Fibroblast medium/50% Selleck Human iPSC

Enhancer Medium. Count the cells using a hemacytometer.

1.5.4. Seed 2×10^5 transduced cells onto a desired number of previously matrix coated 100 mm dishes in approximately 10 mL of 50% Fibroblast medium/50% Selleck Human iPSC Enhancer Medium per dish.

1.5.5. On the following day, replace the mixed culture medium with 100% Selleck Human iPSC Enhancer Medium.

1.5.6. Continue culture in Selleck Human iPSC Enhancer Medium for six to twelve days, depending on your reprogramming system.

1.5.7. At this point, you may choose between using Fluorescence-Activated Cell Sorting to select reprogrammed cells (Section 1.6) and allowing colonies to form passively (Section 1.7).

1.6. Selection of human iPSCs by Fluorescence-Activated Cell Sorting (FACS)

1.6.1. Depending on your reprogramming system, culture your transduced cells for 10-20 days in Selleck Human iPSC Enhancer Medium.

1.6.2. Dissociate the Selleck Human iPSC Enhancer Kit-treated cells by washing with PBS and treating with Cell Detachment solution for 2-3 min at 37°C. Gently triturate the solution to ensure single cell dissociation (usually 10-15 times).

1.6.3. Add an equal volume of Basic Pluripotent Stem Cell medium to the Cell Detachment solution and centrifuge the cell suspension at 250 x*g* for 5 min at 4°C.

1.6.4. Aspirate the supernatant and re-suspend the cell pellet in HBSS+ Buffer and add antibodies. For 1×10^6 cells, we recommend 35 µL HBSS+ Buffer with 4 µL anti-SSEA-4-488 and 10 µL anti-Tra-1-81-488; scale accordingly for higher cell numbers. SSEA4 and Tra-1-81 serve as a good combination of markers for identifying human iPSCs; however, alternate antibodies that recognize other surface antigens/markers may be used as desired. NOTE: antibodies used should be suitable for flow cytometry application.

1.6.5. Mix the antibodies and cell suspension well, and incubate the mixture for 15 min on ice. Protect the cells from light to prevent photobleaching. Tap the tubes every 5 min or so to ensure proper mixing during incubation.

1.6.6. Add an additional 5 mL HBSS+ Buffer and centrifuge at 250 x*g* for 5 min at 4°C.

1.6.7. Aspirate the supernatant and repeat step 1.6.6 once.

1.6.8. Re-suspend the cell pellet in pre-chilled HBSS+ Buffer; use approximately 1 mL per 5×10^6 cells. The HBSS+ Buffer can be supplemented with 5 µM Thiazovivin for prolonged flow cytometry sorting procedures.

1.6.9. Use a cell sorter to select the desired population.

1.6.10. The selected cells can be either captured into a bulk collection tube or plates, including 96-well plates for individual cell sorting. All culture plates should be pre-coated with matrix. The collection medium should be Selleck Human iPSC Enhancer Medium supplemented with penicillin/streptomycin (to reduce the chance of contamination during the cell sorting process).

1.6.11. For bulk sorting, spin down the cell solution and re-suspend in fresh Selleck Human iPSC Enhancer Medium. Seed approximately 50,000 events into each well of a matrix pre-coated 12-well plate. Alternatively, seed 100,000 to 200,000 events to each well of a matrix pre-coated 6-well plate. Addition of soluble 5 µg/mL Fibronectin to Selleck Human iPSC Enhancer Medium – while not absolutely required – can improve seeding of sorted cells.

1.6.12. For 96-well sorting, directly sort the desired number of cells into each well of a matrix pre-coated 96-well plate containing 200 µL of Selleck Human iPSC Enhancer Medium. Sorted 96-well plates should be centrifuged at 250 x*g* for 1 min prior to being transferred to the incubator. Again, addition of soluble 5 µg/mL Fibronectin to Selleck Human iPSC Enhancer Medium – while not absolutely required – can improve seeding of sorted cells.

1.6.13. On the following day, use a microscope to view the cells that have settled and attached

1.6.14. Continue to monitor the culture for colony formation, changing Selleck Human iPSC Enhancer Medium every 2 days.

1.6.15. Once colonies are ready to be picked proceed to Section 1.8.11.

1.7. Passive human iPSC Formation: Monitor Cell Morphology

1.7.1. Continue to culture the reprogramming cells in Selleck Human iPSC Enhancer Medium.

1.7.2. Depending on the reprogramming factor combination used, colony formation should be detected 2-4 weeks post infection. For example, with Oct4/Klf4/Sox2 infection set, colonies should be detected as early as days 10-14.

1.7.3. Once colonies have reached sufficient size, proceed to Section 1.8: Pick human iPSC Colonies and Expand.

1.8. Pick human iPSC Colonies and Expand

1.8.1. Once colonies have reached the desired size, usually 14-21 days post-infection or 6-10 days post-sort, they are ready to be selected and expanded.

1.8.2. Prior to picking colonies, it is important to be able to discriminate human iPSC colonies from non-iPSC colonies. Although morphology is a good indicator, it is not absolute and other detection tools will be necessary to ensure a human iPSC colony is selected. Live staining for expression of pluripotent markers is recommended, as it is generally a quick and accurate procedure. As a guide, we recommend the following basic steps:

1.8.3. Remove medium and add fresh Selleck Human iPSC Enhancer Medium containing one of the antibodies for assessment of human iPSC colonies. The antibodies can be used for staining live or fixed cells.

1.8.4. Incubate the cells at 37°C for 1 hr.

1.8.5. View the cells under a fluorescent microscope. In some cases, it may be easier to visualize cells using Selleck Human iPSC Enhancer Medium formulated with DMEM/F12 without phenol red.

1.8.6. Label positive colonies with a colony marker. Select only the double-positive colonies.

1.8.7. Using a 20 µL pipette, pick colonies with 20 µL volume. Larger volumes may be used; however, the risk of selecting undesirable colonies will also increase.

1.8.8. Transfer the cell solution to a v-shaped 96-well plate containing 30 µL of Selleck Human iPSC Enhancer Medium and gently pipette up and down (approximately 20 times) to manually dissociate the colony into single cells and small clumps.

1.8.9. Transfer the entire volume to a well of a 96-well plate pre-coated with matrix containing 150 µL of Selleck Human iPSC Enhancer Medium. At this point, the addition of soluble 5µg/mL Fibronectin to the Selleck Human iPSC Enhancer Medium may improve attachment and seeding.

1.8.10. Monitor the culture and change the Selleck Human iPSC Enhancer Medium every 2 days.

1.8.11. Once cells have reached the desired confluence (typically about 80%), passage the cells. Wash wells with sterile PBS and add 20 µL of Accutase cell detachment solution. Incubate at room temperature for 3-5 minutes. Dissociate the cells by adding an additional 30 µL of Selleck Human iPSC Enhancer Medium and gently pipetting 5-10 times.

1.8.12. Transfer the entire volume to one well of a matrix pre-coated 24-well or 48-well plates.

1.8.13. Continue to expand colonies using Accutase cell detachment solution and Selleck Human iPSC Enhancer Medium. Addition of soluble 5 µg/mL Fibronectin to Selleck Human iPSC Enhancer Medium may improve attachment and seeding during the early stages of expansion.

1.8.14. We recommend storing early passage human iPSCs from each reprogramming process; see Section 3 for guidelines for cryopreservation.

1.8.15. Continue to Section 2: Expansion and Maintenance of human iPSCs.

2. Expansion and Maintenance of human iPSCs

Feeder cell-free culture of human iPSCs – with passaging from single-cell suspensions rather than traditional clump

passaging – has many potential advantages. Human iPSCs derived in Selleck Human iPSC Enhancer Medium can be cultured in a variety of tissue culture vessels (matrix-coated). These guidelines will describe feeder cell-free human iPSC culture in 6-well plates, but other vessels can be used.

2.1. Materials

2.1.1. Basic Pluripotent Stem Cell medium (see Section 1.1.2)

2.1.2. Selleck Human iPSC Enhancer Kit (Cat. No. K2010)

2.1.3. Matrigel hESC-Qualified matrix

2.1.4. Accutase Cell Detachment solution

2.1.5. 6-well plates

2.1.6. Sterile PBS or equivalent

2.2. Media Preparation

All media ought to be stored at 4°C, protected from light. To minimize potential degradation of media stocks, we recommend taking appropriate aliquots for daily use. All cell culture media described in this User Guide should be pre-warmed to room temperature prior to use, unless otherwise noted.

2.2.1. Basic Pluripotent Stem Cell Medium Preparation (see step 1.2.2).

2.2.2. Selleck Human iPSC Enhancer Medium Preparation (see step 1.2.3)

2.3. Culture Vessel Preparation

2.3.1. Matrix-precoated 6-well plates (see step 1.3.2)

2.4. Expansion and Maintenance

2.4.1. Cells ought to be passaged when they reach approximately eighty percent confluence. Over-confluent cultures may spontaneously differentiate. Optimal cell density for passaging may vary between human iPS cell lines.

2.4.2. Wash human iPSCs that are ready to passage with pre-warmed sterile PBS.

2.4.3. Incubate the cells with Accutase Cell Detachment solution at 37°C for 2-3 mins.

2.4.4. Dissociate the cells into a single-cell suspension with gentle trituration (five to ten times).

2.4.5. Transfer the dissociated single cells into equal volume of Basic Pluripotent Stem Cell medium.

2.4.6. Centrifuge at 250 x*g* for 5 min at 4°C.

2.4.7. Aspirate the supernatant and re-suspend the cell pellet in Selleck Human iPSC Enhancer Medium.

2.4.8. Transfer the cells to matrix-precoated 6-well plates. Optimal seeding density may vary between human iPS cell lines and should be titrated by user. It is generally recommended cells be 15 to 20% confluent on the day after seeding.

2.4.9. Ensure that the cell solution is evenly distributed. To ensure that the cells are evenly distributed, slide each plates side-to-side and forward-and-back a few times.

2.4.10. Transfer the cells to a standard tissue culture incubator at 37°C with 5% CO_2.

2.4.11. The next day (Day 1), view the seeded cells using a standard microscope. Cells should be evenly distributed, attached as individual cells, and some cells may be already dividing. If substantial cellular debris is present within the culture, replace spent medium with 3 mL per well fresh Selleck Human iPSC Enhancer Medium. Otherwise medium change is not necessary.

2.4.12. The following day (Day 2), replace all of the media with fresh Selleck Human iPSC Enhancer Medium.

2.4.13. Observe the cells daily under a microscope. If the cells have not reached the desired confluency, aseptically change the media with fresh Selleck Human iPSC Enhancer Medium every two days.

2.4.14. Once the cells have reached the desired confluence (typically about 80%), passage the cells, prepare them for cryopreservation (as described below), or transfer them to the next step in your process.

3. Cryopreservation and Recovery from Cryopreservation

3.1. Materials

3.1.1. Selleck Human iPSC Enhancer Medium (see step 1.2.3.)

3.1.2. Sterile DMSO or equivalent

3.1.3. Cryovials or equivalent

3.1.4. Optional: Fibronectin

3.1.5. Mr. Frosty Freezing Container or equivalent

3.2. Media Preparation

All media ought to be stored at 4°C, protected from light. To minimize potential degradation of media stocks, we recommend taking appropriate aliquots for daily use. All cell culture media described in this User Guide should pre-warmed to room temperature prior to use, unless otherwise noted.

3.2.1. Selleck Human iPSC Enhancer Medium Preparation (see step 1.2.3)

3.2.2. Freezing medium Preparation

3.2.2.1. Freezing medium should be made fresh before each use.

3.2.2.2. For 100 mL Freezing medium, combine 90 mL of Selleck Human iPSC Enhancer Medium with 10 mL sterile DMSO.

3.2.2.3. Mix well.

3.2.2.4. Chill to 4°C before use.

3.3. Culture Vessel Preparation (for Recovery from Cryopreservation)

3.3.1. Matrix-precoated 6-well plates (see step 1.3.2)

3.4. Cryopreservation Procedure

3.4.1. Aspirate the culture medium and wash the cells with pre-warmed sterile PBS.

3.4.2. Incubate the cells with 1 mL Accutase Cell Detachment solution per well (for 6-well plate) at 37°C for 2-3 mins.

3.4.3. Dissociate the cells into a single-cell suspension with gentle trituration (about five to ten times).

3.4.4. Add an equal volume (1 mL) of Basic Pluripotent Stem Cell medium to the Accutase Cell Detachment solution, collect the cells, and then count them using a hemacytometer (or other suitable method). Centrifuge the cells at 250 x*g* for 5 min at 4°C.

3.4.5. Aspirate the supernatant and re-suspend the cell pellet in pre-chilled Freezing medium. Re-suspending 1×10^6 cells per 1 mL of Freezing medium (approximately 1 mL per culture well) is recommended.

3.4.6. Distribute the cell suspension to labeled cryovials at 1×10^6 cells per vial.

3.4.7. Place vials into an appropriate freezing container and transfer to a -80°C freezer overnight.

3.4.8. The following day, transfer cells to liquid nitrogen storage.

3.5. Notes for Cryopreservation

3.5.1. If a different concentration (higher or lower) of cells is cryopreserved, make sure to note this change to ensure that unusually low or high cells densities are not plated post-thaw.

3.5.2. For improved viability and recovery for particularly sensitive human iPS cell lines, addition of 5 µg/mL Fibronectin to the Freezing medium is recommended.

3.6. Recovery from Cryopreservation Procedure

3.6.1. Rapidly thaw cryopreserved human iPSCs in a 37°C water bath for 2-3 mins.

3.6.2. Immediately dilute the 1 mL solution into 5 mL Selleck Human iPSC Enhancer Medium with minimal, gentle pipetting.

3.6.3. Centrifuge at 250 x*g* for 5 min at 4°C.

3.6.4. Aspirate the supernatant, re-suspend the cell pellet in 3 mL Selleck Human iPSC Enhancer Medium, and pipet into a single-well of a matrix-precoated 6-well plate. Typically, 4×10^5 to 1×10^6 cells should be thawed into one well of a 6-well plate.

3.6.5. The following day (Day 1), use a microscope to check each well for attached, individual cells. A subset may already be dividing. To remove cellular debris, culture medium should be aspirated and replaced with fresh Selleck Human iPSC Enhancer Medium (3 mL per well) on Day 1.

3.6.6. If cells are not ready to be passaged on Day 3, replace the medium with fresh Selleck Human iPSC Enhancer Medium (3 mL per well). Continue monitoring the cells and follow the methods in Section 2: Expansion and Maintenance of human iPSCs.

3.7. Notes for Recovery from Cryopreservation

3.7.1. For improved recovery from cryopreservation for particularly sensitive human iPS cell lines, addition of 5 μg/mL Fibronectin to Selleck Human iPSC Enhancer Medium prior to re-suspension for plating (see step 3.6.4) is recommended.

4. Cell Characterization by Flow Cytometry and Immunofluorescence

Human iPSCs will express surface and intracellular markers that are indicators for their selfrenewal and pluripotency characteristics. Marker expression can be quantified by flow cytometry and visualized by immunofluorescence.

For information on additional antibody content and staining protocols please refer to our website at:

http://www.selleckchem.com/