Simplicon™ Human iPS Cell Line

Stem Cell Line

Cat. # SCC271

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.
NOT FOR HUMAN OR ANIMAL CONSUMPTION.

pack size: ≥ 1X10^6 cells/vial

Store in Liquid Nitrogen

Data Sheet

Description

The discovery that somatic cells could revert back to pluripotent like cells in 2006 by Shinya Yamanaka created an entirely new area of stem cell biology. IPS cells removed the ethical concerns associated with embryonic stem cells and allowed scientists to model human diseases that were previously impossible to model such as Alzheimer's, Parkinson's and Autism. Human induced pluripotent stem cells (iPSCs), have the capacity to give rise to differentiated progeny representative of all three germ layers (ectoderm, endoderm, and mesoderm). The ability to expand pluripotent cells *in vitro* and subject them to direct differentiation to produce specific cell types is crucial for the development of potential cell-based therapies and disease-in-a-dish cell models for research.

Simplicon™ RNA Reprogramming Technology is a next generation non-integrating reprogramming system that uses a single synthetic self-replicating RNA strand engineered to mimic cellular RNA to generate human iPS cells. The single RNA strand contains the four reprogramming factors, OCT-4, KLF-4, SOX-2 and GLIS1 (OKSG) and enables extremely efficient reprogramming using a single transfection step without any viral intermediates or host genome integration. The Simplicon™ human iPS cell line was generated by reprogramming human foreskin fibrobalsts (SCC058) using the Simplicon™ RNA Reprogramming Kit (SCR550). The cell line is integration-free, highly characterized and maintains a pluripotent phenotype over multiple passages.

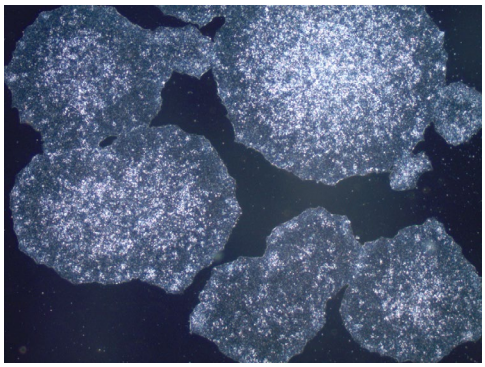

Figure 1. Undifferentiated morphology of Simplicon™ human iPS cells display dense colonies with clear and defined borders.

Storage and Handling

Simplicon™ human iPS cells should be stored in liquid nitrogen. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting the cell marker expression and functionality.

Quality Control Testing

- Each vial contains ≥1X10^6 viable cells.
- Cells are tested negative for HPV-16, HPV-18, Hepatitis A, B, C, and HIV-1 & 2 viruses by PCR.
- Cells are negative for mycoplasma contamination.
- Each lot of cells is genotyped by STR analysis to verify the unique identity of the cell line.

References

1. Dowdy, SF, et al. Efficient generation of human iPSCs by a synthetic self-replicative RNA. Cell Stem Cell. 2013 Aug 1;13(2):246-54.
2. Clark, AT, et al. An integration-free, virus-free rhesus macaque induced pluripotent stem cell line (riPSC89) from embryonic fibroblasts. Stem Cell Res. 2016 Sep;17(2):444-447.
3. Elvassore, N, et al. Microfluidic reprogramming to pluripotency of human somatic cells. Nat Protoc. 2019 Mar;14(3):722-737.
4. Rabelink, TJ, et al. Renal Subcapsular Transplantation of PSC-Derived Kidney Organoids Induces Neo-vasculogenesis and Significant Glomerular and Tubular Maturation *In Vivo*. Stem Cell Reports. 2018 Mar 13;10(3):751-765.

Ver 1.0/2019-05-02/SCC271/NJA

SPECIES LEGEND: **H** Human **Ca** Canine **M** Mouse **R** Rat **Rb** Rabbit **B** Bovine **P** Porcine **WR** Most Common Vertebrates

Please visit www.millipore.com for additional product information and references.

Results

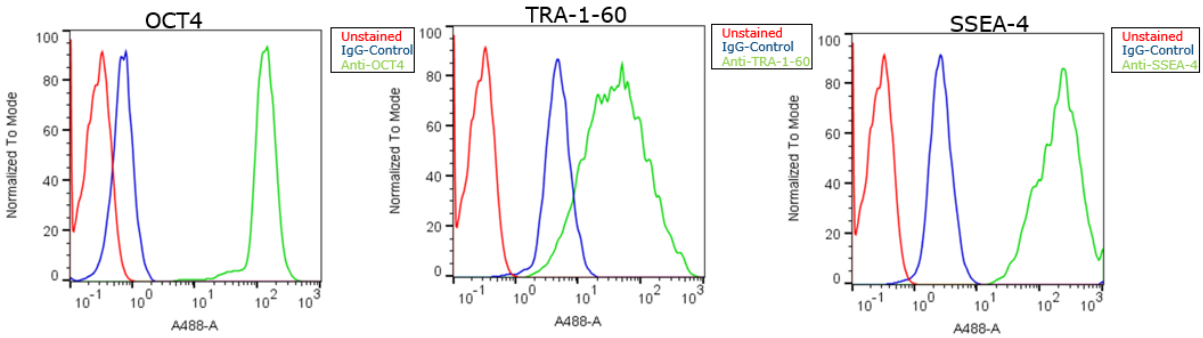

Figure 2. Characterization of Simplicon™ human iPS cells using flow cytometry. The Simplicon human iPS cells are >90% positive for pluripotency markers Oct-4, TRA-1-60 and SSEA-4.

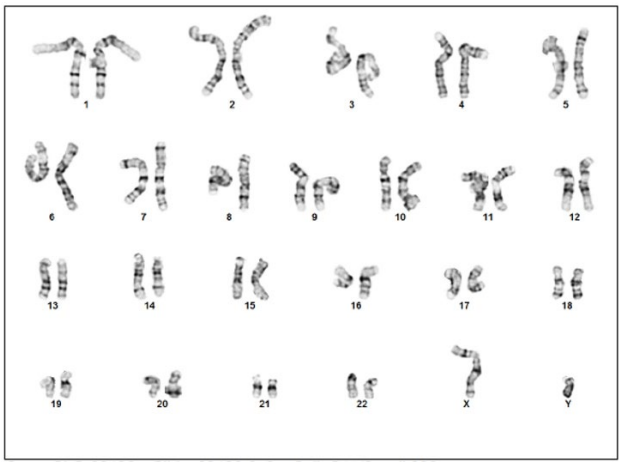

Figure 3. Chromosomal analysis of Simplicon™ human iPS cells. Karyotype of human iPSCs show normal male karyotype with no chromosomal abnormalities.

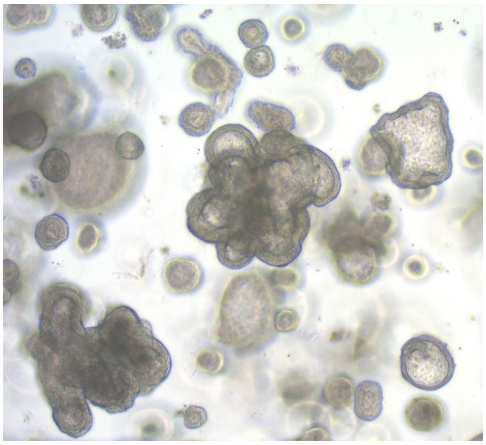

Figure 4. Differentiation potential of Simplicon™ human iPS cells. Human iPSCs were differentiated into colonic organoids following protocol outlined by Crespo et. al.

Stem Cell Qualified ECM Gel Coating:

Human iPS cells should be thawed into tissue culture-treated plates coated with 1:20 dilution of Stem Cell Qualified ECM Gel (Cat. No. CC131-5ML). Generally, one cryovial may be thawed into 1-2 wells of a ECM Gel coated 6-well plate. Below are general guidelines for the coating of 6-well plates and culture flasks with Stem Cell Qualified ECM Gel.

1. Thaw Stem Cell Qualified ECM Gel on ice. Keep on ice and use pre-cooled medium and pipettes to avoid gelling of the ECM gel**. IMPORTANT: Do not thaw at temperatures higher than 15°C to avoid gelling.**

2. Dilute the ECM Gel 1:20 with cold DMEM/F12 medium. For example, to every 0.5 mL ECM Gel, add 9.5 mL cold DMEM/F12 medium for a total volume of 10 mL. Scale according to the volumes required.

3. Cover the cultureware with 1:20 ECM Gel solution (1.5 mL per well of 6-well plate). Incubate at 2-8°C overnight or at room temperature for 30 minutes before use. If not used immediately, store coated cultureware at 2-8°C for up to 1 week.

4. On the day of thawing, acclimate ECM Gel coated plates for 15-20 min at room temperature. Remove the ECM Gel coating. Add 2 mL human ES/iPS medium to each well. Set plate aside until cells are ready to be thawed or passaged. **IMPORTANT: Do not allow the flask to dry out.**

Thawing:

1. Remove the vial of cryopreserved cells from liquid nitrogen storage and quickly thaw the cells in a 37°C water bath. Closely monitor until only small ice crystals remain. Quickly remove the vial from the waterbath. **IMPORTANT: Do not vortex the cells or leave them in the water bath for too long.** Disinfect the outside of the vial with 70% ethanol or isopropanol.

2. In a laminar flow hood, use a 1 or 2 mL pipette to transfer the cells to a sterile 15 mL conical tube. Be careful not to introduce any bubbles during the transfer process.

3. Using a 10 mL pipette, slowly add dropwise 9 mL of Human ES/iPS Medium to the 15 mL conical tube. **IMPORTANT: Do not add the whole volume of media at once to the cells. This may result in decreased cell viability due to osmotic shock**. Gently mix the cell suspension by slow pipeting up and down twice. Be careful not to introduce any bubbles. IMPORTANT: Do not vortex the cells.

4. Centrifuge the tube at 300 x g for 5 minutes at room temperature (15 – 25°C). Aspirate the supernatant. Resuspend the cell pellet in 1 mL of Human ES/iPS medium by gently pipetting the cells up and down twice. Take care to maintain the cells as aggregates.

5. Transfer 1mL of the thawed cell aggregates to one well of the ECM Gel coated 6-well plate containing 2 mL Human ES/iPS medium that had been set aside. Total volume per well = 3 mL.

6. Place the plate in a 37°C incubator. Agitate the plate **gently** from side to side and forward and backwards to ensure that the cell aggregates are evenly distributed across the surface of the well. Incubate in a 37°C, 5% CO_2 incubator.

7. After 10 – 15 minutes, visually inspect the plate to ensure that newly thawed cell aggregates are evenly distributed across the surface of the wells. Plates that have not been properly agitated may have cell clumps aggregating toward the center of the wells. This uneven distribution at the center may later cause spontaneous differentiation of human ES/iPS cells. In the event clumps are not evenly distributed, agitate the plate gently from side to side and forward and backwards for a longer extended time.

8. The next day, replace with 3 mL per well of fresh Human ES/iPS medium. Monitor and exchange with 3 mL fresh medium daily. For weekend, use 4 mL media on Friday to sustain cells over the weekend.

EZ-LiFT™ Passaging Without a Shaker: (Cat. No. SCM139-100ML)

The protocol is based on 1 well of a 6-well plate. Do not passage more than 3 wells at a time. For additional information, refer to SCM139-100ML datasheet.

1. Start with high-quality human ES or iPS culture that is 60-80% confluent. Warm EZ-LiFT™ Reagent to 37°C before starting.
Critical Note: *Do not use ice-cold EZ-LiFT™ Reagent as this will slow down the colony dissociation.*

2. Aspirate the culture medium and wash wells twice with 1.5 mL EZ-LiFT™ Reagent. Aspirate after each wash.

3. Add 1 mL of EZ-LiFT™ reagent to each well. Incubate the plate at 37°C for 4 minutes.

4. After 4 minutes, tap rapidly on the bottom of the plate (i.e. 20-25 taps in 5 secs).

5. Place the plate back in the 37°C incubator for an additional 4 minutes.

6. After 4 minutes, tap rapidly on the bottom of the plate (i.e. 20-25 taps in 5 secs).
Important: Do not rinse the wells.

7. Perform a quick microscopic inspection of the well(s).
    a. If a significant number of detached clumps are visible, proceed to step 8.
    b. If no obvious detachment is observed, repeat steps 4-7 except that in step 5, the 37°C incubation should be for 2 instead of 4 minutes. Proceed to step 8.

8. Gently collect the cell suspension (~1 mL) and transfer to a 15 mL conical tube. Neutralize with 5 mL of culture medium by gently adding the medium to the cell suspension. **Do not pipette up and down as this may break cell clumps into single cells suspension.**

9. Centrifuge at 800 rpm or 130 x g for 3 minutes. Aspirate the supernatant.

10. Gently resuspend the cell pellet in 1 mL pluripotent medium (i.e. mTeSR or PluriSTEM).
Caution: Do not pipette up and down more than two times. Over-pipetting may result in single cell dissociation.

11. Passage dissociated cell clumps to newly coated 6 well plates. If you are a first time user, we recommend passaging at a conservative 1:5 split ratio. After becoming familiar with the protocol, the split ratio may be increased to 1:9 up to 1:30 split ratio. Monitor cells daily. Newly passaged ES/iPS cells will typically reach 60-80% confluence in 6-8 days depending upon the split ratio.

EZ-LiFT™ Passaging Using a Shaker:

Important Note: The protocol was developed using a Labnet VorTemp™ 56 orbital shaker (National Labnet Co. Inc., Woodbridge, NJ) with 3 mm shaking orbit. The protocol is based on 1 well of a 6-well plate. Do not passage more than 3 wells at a time.

1. Start with high-quality human ES or iPS culture that is 60-80% confluent. Warm EZ-LiFT™ Reagent to 37°C before starting.
Critical Note: *Do not use ice-cold EZ-LiFT™ Reagent as this will slow down the colony dissociation.*

2. Place a Labnet VorTemp™ 56 orbital shaker into the 37°C incubator. Be sure to wipe down the shaker with 70% ethanol first.

3. Aspirate the culture medium and wash wells twice with 1.5 mL EZ-LiFT™ Reagent. Aspirate after each wash.

4. Add 1 mL of EZ-LiFT™ reagent to each well. Seal the plate with paraffin to prevent cross-contamination while shaking.

5. Shake plate at 500 rpm or 0.42 – 0.51 x g for 5 minutes at 37°C. After 5 minutes, tap rapidly on the bottom of the plate (i.e. 20-25 taps in 5 secs). **Important**: Do not rinse the wells.

6. Perform a quick microscopic inspection of each well.
    c. If a significant number of detached clumps are visible, proceed to step 7.
    d. If no obvious detachment is observed:
        1. Tap rapidly on the bottom of the plate (i.e. 20-25 taps in 5 secs).
        2. Shake for 1 minute
        3. Tap rapidly on the bottom of the plate (i.e. 20-25 taps in 5 secs). Procced to Step 7.

7. Gently collect the cell suspension (~1 mL) and transfer to a 15 mL conical tube. Neutralize with 5 mL of culture medium by gently adding the medium to the cell suspension. **Do not pipette up and down as this may break cell clumps into single cells suspension.**

8. Centrifuge at 800 rpm or 130 x g for 3 minutes. Aspirate the supernatant.

9. Gently resuspend the cell pellet in 1 mL pluripotent medium (i.e. mTeSR or PluriSTEM).
Caution: Do not pipette up and down more than two times. Over-pipetting may result in single cell dissociation.

10. Passage dissociated cell clumps to newly coated 6 well plates. If you are a first time user, we recommend passaging at a conservative 1:5 split ratio. After becoming familiar with the protocol, the split ratio may be increased to 1:9 up to 1:30 split ratio. Monitor cells daily. Newly passaged ES/iPS cells will typically reach 60-80% confluence in 6-8 days depending upon the split ratio.

Cell Cryopreservation:

1. Start with high-quality human ES or iPS culture that is 60-80% confluent. Treat wells with EZ-LiFT™ as outlined in the protocols above. However instead of passaging to newly coated 6-well plates, resuspend cell pellet in 1 mL pluripotent media (i.e. mTeSR1 or PluriSTEM) containing 10% DMSO. **Caution:** Do not pipette up and down more than two times. Over-pipetting may result in single cell dissociation.

2. One well of EZ-LiFT™ treated cells may be frozen into 3-5 cryovials. Depending upon the desired number of cryovials, add the requisite volume of pluripotent media containing 10% DMSO. For example, to obtain 5 cryovials, add 4 mL pluripotent media containing 10% DMSO to the 1 mL cell suspension in step 1.

3. Transfer cryovials to Nalgene slow freeze Mr. Frosty container and store at -80°C.

4. After 24 hours, transfer cryovials to liquid nitrogen for long-term storage.

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