Data Sheet

Simplicon® Human iPS Cell Line

Stem Cell Line

SCC271

Pack Size: ≥ 1X10^6 cells/vial

Store in Liquid Nitrogen.

FOR RESEARCH USE ONLY

Not for use in diagnostic procedures. Not for Human or Animal Consumption.

Background

The discovery that somatic cells could revert to pluripotent like cells in 2006 by Shinya Yamanaka created an entirely new area of stem cell biology. Human 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.

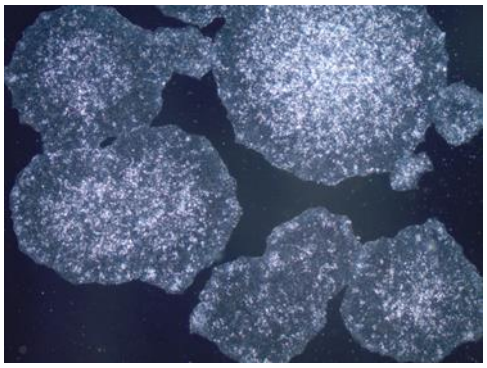

Figure 1: Undifferentiated morphology of Simplicon® human iPS cells display dense colonies with clear and defined borders.

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 and 2 viruses by PCR.
- Cells are negative for mycoplasma contamination.
- Each lot of cells are genotyped by STR analysis to verify the unique identity of the cell line.

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.

Representative Data

Results

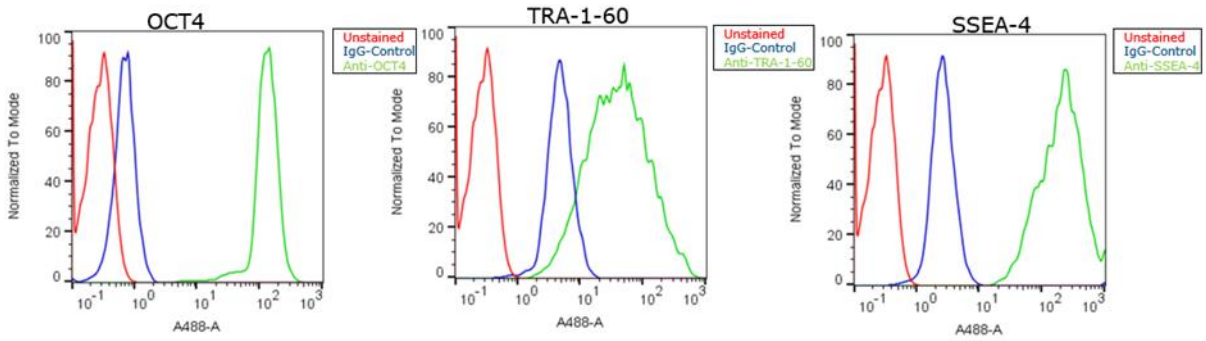

Figure 2: Characterization of Simplicon® human iPS cells using flow cytometry. The Simplicon human iPS cells are >90% positive for pluripotency markers Oct-4, TRA-1-60 and SSEA-4.

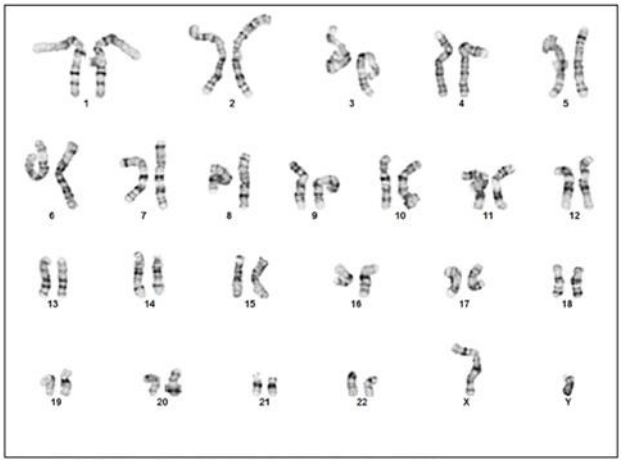

Figure 3: Chromosomal analysis of Simplicon® human iPS cells. Karyotype of human iPSCs show apparently normal male karyotype with no chromosomal abnormalities.

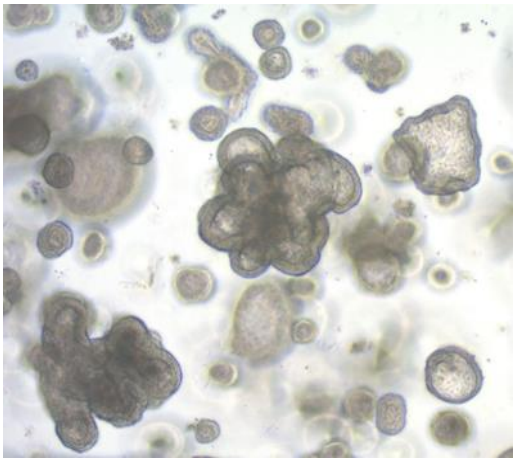

Figure 4: Differentiation potential of Simplicon® human iPS cells. Human iPSCs were differentiated into colonic organoids using kits SCM301, SCM303 and SCM304.

Protocols

Culture Media Recommendation

Human iPS cells can be cultured in mTeSR-1 media (85850). Refer to manufacturing datasheet to prepare these media appropriately.

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 (CC131-5ML). Generally, one cryovial may be thawed into 1 well of an 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 Complete mTeSR media 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 water bath.

   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 pipetting up and down twice. Be careful not to introduce any bubbles. Do not vortex the cells.
4. Centrifuge the tube at 218 x *g* for 5 minutes at room temperature (15-25 °C). Aspirate the supernatant. Resuspend the cell pellet in 1 mL of mTeSR media by gently pipetting the cells up and down twice. Take care to maintain the cells as clumps.
5. Transfer 1mL of the thawed cell clumps to one well of the ECM Gel coated 6-well plate containing 2 mL mTeSR media that had been set aside. Total volume per well =3 mL.
6. Agitate the plate gently from side to side and forward and backwards in the TC hood to ensure that the cell clumps are evenly distributed across the surface of the well.
7. Place the plate in a 37 °C incubator. Again, agitate the plate gently from side to side and forward and backwards a few times to ensure clumps are evenly distributed across the surface of the well. Incubate in a 37 °C, 5% CO_2 incubator.

   IMPORTANT: Inadequate agitation of plates can cause cell clumps to accumulate at the center of the wells. This uneven distribution may lead to spontaneous differentiation of human embryonic stem (ES) or induced pluripotent stem (iPS) cells. To address uneven distribution, re-agitate the plate by moving it side to side and forward and backward multiple times in the incubator.
8. The next day, replace with 3 mL per well of complete mTeSR media. Monitor and exchange with 3 mL fresh medium daily. iPSC culture should be ready to be passaged around 5-7 days.

   IMPORTANT: Cells should be fed 4-5 mL of Complete mTeSR media on Friday to sustain cells over the weekend. Only 1 media change is required either on Saturday evening or Sunday by noon.

Enzymatic Passaging with PluriSTEM Dispase II Solution (SCM133)

1. Refer to the Stem Cell Qualified ECM Gel Coating Section above for coating culture plates.
2. Prior to passaging the iPS culture, bring the ECM coated 6-well plate to room temperature in the TC culture hood.
3. Aspirate off the ECM coating solution and add 2 mL of Complete mTeSR media to each well.

   IMPORTANT: Do not allow the plate to dry out.
4. Thaw an aliquot of PluriSTEM Dispase II Solution in room temperature water, disinfect the outside of the tube with 70% IPA and place in the TC culture hood.

   IMPORTANT: PluriSTEM Dispase II Solution is a ready-to-use reagent at 1 mg/mL stock solution. Aliquot stock solution into 10 mL aliquots and stored at 2-8 °C for up to 4 months from date of receipt. Frozen aliquots may be thawed and stored at 2-8 °C for up to 2 weeks. Avoid multiple freeze thaw cycles to maintain proper enzymatic activity.
5. Remove iPSC culture from the incubator and observe under the microscope to check the status of the cells.
6. Move the culture to the TC hood and aspirate off media.
7. Replace with 3 mL of Complete mTeSR media.
8. On the day of passage, remove culture from the incubator and change media in the TC hood.
9. Inspect hiPSC culture under the microscope for spontaneous differentiation.
10. Clean culture of spontaneous differentiation colonies under a dissecting microscope.

    IMPORTANT: Attached a P200 pipet tip without a filter to the tip of a 2 mL aspirating pipet. Scrape away areas of differentiation with it. Be discriminating and scrape away any areas that harbor a hint of differentiation.
11. Transfer the culture to the TC culture hood and aspirate off media.
12. Wash the culture with 2 mL of 1X PBS and aspirate.
13. Add 1 mL of PluriSTEM Dispase II Solution to a well of a 6-well plate.
14. Incubate for 6-8 minutes in the 37 °C incubator. Check at 6 minutes and determine if the colonies require a longer incubation time.

    IMPORTANT: The colonies are ready when the edges of the colonies started to curl up.
15. Aspirate off Dispase II solution from the well.
16. Wash 2 with 2 mL of 1X PBS.
17. Aspirate off 1X PBS.
18. Add 1.5 mL of Complete mTeSR media to the well
19. Gently detach the colonies by scraping horizontally 7-8 times and vertically 7-8 times using the tip of the scraper.
20. Afterward, place the scraper blade directly on the rim of the well and scrape around the inside rim of the well first and then horizontally and vertically to completely dislodge the colonies.
21. Transfer the clumps suspension with a 5 mL pipet into a 15 mL conical tube containing 2 mL of Complete mTeSR media.
22. Add 2 mL of Complete mTeSR media to the well to rinse off residual clumps and transfer to the 15 mL conical tube containing the clumps suspension.
23. Centrifuge the 15 mL conical tube at 218 x *g* for 5 minutes at room temperature.
24. Aspirate off supernatant.
25. Pipet up 1 mL of Complete mTeSR media using a 5 mL pipet and gently tap the pellet with the tip of the pipet 2-3 times to break up the pellet into smaller clumps and then slowly release the 1 mL of Complete mTeSR media. If there are big chunks presence, guide the tip of the pipet to them and tap a couple times to break into smaller clumps.

    IMPORTANT: Avoid breaking the pellet into too small of a clump.
26. Seed the clump suspension at 1:3, 1:6, 1:10 and etc., into each well of a 6-well ECM coated plate to determine which split ratio works the best.

    RECOMMENDATION: use a P200 pipet with a wide-bore tip to transfer the clump suspension into each well by adding dropwise throughout the surface of each well.

27. Gently agitate the plate a few times from side to side and forward and backward to ensure even distribution of clumps across the surface of each well. Check under a microscope to confirm.
28. Place the plate in a 37 °C incubator with 5% CO_2. Again, gently agitate the plate from side to side and forward and backward a few more times to endure even distribution of clumps across the surface of the well.
29. After confirming that the cell clumps are evenly distributed in the well, keep the plate in the incubator overnight.
30. Next day, remove the plate and observe under microscope. There should be clumps that are not attaching and some that are. Change media with 3 mL of Complete mTeSR media and plate back in the incubator.
31. Repeat observing under the microscope and changing media daily.

    IMPORTANT: Cells should be fed 4-5 mL of Complete mTeSR media on a Friday to sustain cells over the weekend. Only 1 media change is required on either Saturday evening or Sunday by noon. Human iPS cells should be matured and ready to passage around days 5-7 after passage.

Cryopreservation Using PluriSTEM Dispase II Solution (SCM133)

When iPS cells reached 80-85% confluency, they are ready to be passaged or cryopreserved. For cryopreservation, it is recommended to freeze an 80-85% confluent well into 1 cryovial in cold freezing media (Complete mTeSR media + 10% DMSO).

1. Passage iPSC culture with Dispase II as described in the Enzymatic Passaging with PluriSTEM Dispase II Solution (SCM133) section.
2. Once the cells have been pelleted, pipet up the appropriate amount of cold freezing media (Complete mTeSR media + 10% DMSO) and tap the pellet to break into appropriately sized clumps.
3. Slowly release the freezing media to the clumps and gently resuspend the suspension 1-2 times.
4. Swirl the tube to mix and transfer 1 mL at a time into a pre-labeled cryovial. Repeat until all are transferred to cryovials.
5. Immediately place cryovials into Mr. Frosty® and store at -80 °C overnight.
6. Transfer cryovials to the liquid nitrogen tank for long-term storage.

EZ-LiFT™ Passaging Without a Shaker: (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).
   - If a significant number of detached clumps are visible, proceed to step 8.
   - 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: (SCM139-100ML)

The protocol was developed using a Labnet VorTemp™ 56 Orbital 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.

   IMPORTANT: 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 the 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 parafilm 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 the 5 minutes, tap rapidly on the bottom of the plate (for example, 20-25 taps in 5 secs).

   IMPORTANT: Do not rinse the wells.
6. Perform a quick microscopic inspection of each well.
   - If a significant number of detached clumps are visible, proceed to step 7.
   - If no obvious detachment is observed:
     - Tap rapidly on the bottom of the plate (for example, 20-25 taps in 5 secs).
     - Shake for 1 minute.
     - Proceed 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.

   IMPORTANT: Do not pipet up and down as this may break cell clumps into single cell suspension.
8. Centrifuge at 800 rpm or 140 x *g* for 3 minutes. Aspirate off the supernatant.
9. Gently resuspend the cell pellet in 1 mL mTeSR media.

   IMPORTANT: Do not pipet 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. For first-time user, we recommend passaging at a conservation 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 Using EZ-LiFT™

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.

References

1. Dowdy SF, et al. Efficient generation of human iPSCs by a synthetic self-replicative RNA. Cell Stem Cell. 2013;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;17(2):444-447.
3. Elvassore N, et al. Microfluidic reprogramming to pluripotency of human somatic cells. Nat Protoc. 2019;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;10(3):751-765.

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Document Template 20306518 Ver 6.0
20401665 Ver 4.0, Rev 26MAY2025, AV