**Recommended Dissociation Protocol**

1. Warm an aliquot of Stem Cell Dissociation Reagent working solution to room temperature.
2. Aspirate and discard the stem cell culture medium.
3. Rinse the cells once with 5 mL of DMEM:F12 (ATCC 30-2006) per 6-cm dish.
4. Add 3 mL of Stem Cell Dissociation Reagent working solution to the dish.
5. Incubate at 37°C for 10 to 15 minutes or until the borders of the individual colonies begin to loosen and fold back. View the dish under the microscope starting at 5 minutes as incubation time may vary depending on the cell line and colony size.
6. Aspirate the Stem Cell Dissociation Reagent and gently rinse the colonies with 5 mL of DMEM: F12 Medium, taking care not to dislodge the cells during manipulation.
7. Add 3 mL of stem cell culture medium to the dish, and detach the cells by pipetting up and down 3-4 times with a 1 mL tip. Take care not to overpipette the culture into a singlecell suspension as single cells will not establish colonies after seeding.
8. Transfer the cell aggregates to a 15 mL conical tube.
9. Add an additional 3 mL of stem cell culture medium to the dish to collect any remaining cells. Transfer this rinse to the 15 mL conical tube containing the cell aggregates.
10. Centrifuge the cell aggregates at 200 x g for 5 minutes.
11. Aspirate the supernatant and discard. Gently tap the bottom of the tube to loosen the cell pellet.

**Cryopreservation Protocol**

1. Detach stem cell colonies from the dish as described in the recommended dissociation protocol.
2. Remove the Stem Cell Freezing Media from storage and swirl to mix. Keep cold. Decontaminate by dipping in or spraying with 70% alcohol.
3. Add 2 mL of cold Stem Cell Freezing Media to the tube containing the cell pellet. Gently resuspend the pellet by pipetting up and down 5-6 times with a 1 mL tip, maintaining the cell aggregates.
4. Immediately transfer 1 mL each of the cell suspension into two labeled cryovials.
5. Freeze the cells gradually at a rate of -1°C/min until the temperature reaches -70°C to -80°C. A
Handling Procedure for Frozen Cells and Initiation of Cultures

1. 30 Minutes Prior to Handling Cells - Pre-warm the appropriate stem cell culture medium at 37°C for at least 30 minutes before adding to cells.
2. Remove cryovial of frozen cells from liquid nitrogen.
3. Thaw the cells by gentle swirling in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 1 to 2 minutes). Remove the cryovial from the water bath when only a few ice crystals are remaining.
4. Sterilize the cryovial by rinsing with 70% ethanol.
5. Using a 1 mL or 5 mL pipette, gently transfer the cell suspension to a 15 mL conical tube.
6. Slowly add 4 mL stem cell culture medium dropwise, to the conical tube. Use an additional 1 mL of media to rinse the cryovial and transfer the liquid to the 15 mL conical tube. Shake the conical tube gently to mix the cells while adding media.
7. Gently pipette the cells up and down several times to mix thoroughly. Avoid breaking apart the aggregates into a single-cell suspension.
8. Centrifuge the cells at 200 x g for 5 minutes.
9. Aspirate the supernatant and discard. Gently tap on the bottom of the tube to loosen the cell pellet.
10. Add 1 mL of stem cell culture medium that has been supplemented with ROCK Inhibitor Y27632 (ATCC ACS-3030) to a final concentration of 10 µM. Gently resuspend the pellet by pipetting up and down 5 to 6 times with a 1 mL tip, maintaining the cell aggregates.
11. Plate the cells as desired under feeder-dependent or feeder-free culture conditions. The presence of 10 µM ROCK Inhibitor Y27632 in the stem cell culture medium is recommended.

Quality Control Specifications
Stem Cell Freezing Media is tested for pH, appearance, and sterility. Recovery, morphology and differentiation of hESCs and hiPSCs are confirmed after cryopreservation and thawing. A Certificate of Analysis is available upon request.

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