Freezing Human Pluripotent Stem Cells in Feeder-Free Conditions

Purpose: This protocol describes the cryopreservation process for human pluripotent stem cells cultured with Matrigel and TeSR in a 6 well plate format.

Materials:

- Human pluripotent stem cells
- Cryopreservation Media (50% TeSR, 30% KOSR, 20% DMSO)
- TeSR w/30% KOSR
- Versene EDTA
- PBS(-/-)
- DMEM-F12
- Labeled Cryovials
- 15 and 50mL tubes

Procedure:

1. Mark areas of differentiation using the objective marker or pen.
2. Aspirate off the spent media and aspirate marked areas of differentiation in the well using the aspirating tip. Remember to change the tip between different cell lines to avoid cross contamination.
3. Wash each well with 1mL PBS. Gently add the PBS and other liquid reagents by pipetting against the edge of the well.
4. Add 1mL of room temperature Versene EDTA to each well and incubate at 37°C for 6-9 minutes. Incubation times will vary based on cell density.
5. During the incubation step, aspirate the DMEM-F12/Matrigel solution from the previously coated matrigel plate and add 2mL of TeSR media per well.
6. After incubation, check the cells under a microscope. Cells should appear shiny and separated with small gaps or holes appearing inside colonies. Cells should still be attached to the surface. In some cases, colonies will have slightly curled edges and minimal amounts of floating cells/debris will be visible.
7. When cells appear as mentioned in step 6, gently aspirate Versene EDTA from the well without disturbing the loosely attached cells.
8. Add 1mL of TeSR w/30% KOSR to each well and gently pipette to remove cells from the growth surface.
9. Collect cell solution and transfer to a 50mL tube.
10. Add cryopreservation media drop wise to cell solution. 1mL of cryopreservation media is used from every 1mL of cell solution. Example: if freezing 3 wells from a 6 well plate you will have 3mL of cell solution. 3mL of cryopreservation media will be added for 6mL total. The final DMSO concentration will be 10%.
11. Aliquot 1mL of cell/cryopreservation media solution into labeled cryovials.
12. Cap cryovials and transfer to a Styrofoam freezing rack.
13. Place Styrofoam rack in -80°C freezer overnight. Transfer to liquid nitrogen cryostorage the following day. Foam freezing racks and similar cell freezing containers (like Nalgene's Mr. Frosty) allow for a continuous cooling rate of -1°C/min. Slow continuous cooling in

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combination with cyroprotectants (DMSO) prevents the formation of cell damaging ice crystals.

Troubleshooting: -80°C freezers are not suitable long-term storage solutions for frozen cell stocks. Prolonged storage at -80°C will result in reduced cell viability. Transfer frozen cell stocks to a liquid nitrogen cryostorage system within 1 week. Always map the box position of cell stocks when transferring to liquid nitrogen storage,

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