SOP iPSC PT04-2v1	Title: **Culture and Maintenance of Pluripotent Stem Cells on matrigel and mTeSR1 culture media**

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OBJECTIVE

This SOP describes the culture and maintenance of established Pluripotent Stem Cells (PSC) cell lines on matrigel coating and mTeSR1 culture media

DOCUMENTATION

SOP iPSC PT04-1v1 Cryopreservation and Recovery Upon thaw of PSCs on matrigel and mTeSR1 culture media
SOP iPSC PT02-1v1 Preparation of mTeSR1 Media for the maintenance of PSC
SOP iPSC PT02-2v1 Preparation of Matrigel for Maintenance of PSCs

MATERIAL

Equipment

- Class II Microbiology Safety Cabinet
- Centrifuge
- Incubator (37ºC ± 0.5ºC/5% ± 0.5% CO_2)

Consumables

- Matrigel (Corning BV, Catalog #356234)
- mTeSR1 culture media
- Phosphate buffered saline (PBS) without magnesium and calcium (Cultek, SLU, Catalog #17-516F)
- EDTA 0.5M (Life Technologies, Catalog #AM9260G)
- 10mm dish (Corning, Catalog #430167)
- 6 well plates (Corning, Catalog #353-046)
- 50 mL centrifuge tubes
- 15 mL centrifuge tubes
- 5mL/10mL/25mL/50mL stripettes
- P100/P200/P20 pipettes

PROCEDURE

NOTE: All following cell manipulations, tissue culture vessel preparations and media preparations must be performed under aseptic conditions within a microbiological safety cabinet.

NOTE: the microbiological safety cabinet must be cleaned thoroughly by wiping all base surfaces with 70% alcohol.

1. Daily media exchange with mTeSR1 (see SOP iPSC PT02-1v1)
2. PSC lines must be observed daily for PSC-like morphology, the presence of differentiated cells and confluence. Using these observations, the operator must determine if the cells require further action (passage, cryopreservation or preparation for testing).

Chemical passaging of human PSCs using EDTA

NOTE: Cell lines should be passaged when the cells are approximately 80-85% confluent and in their log phase of growth.

NOTE: Split ratios must be determined from typical cell line growth patterns and must be adjusted to ensure that cells are passaged within 4-5 days of culture. Generally, sparse cultures must be split at lower ratios (1:1-1:4) and dense cultures at higher ratios (1:4-1:8).

3. Prepare 0.5mM EDTA by adding the appropriate volume of 0.5M EDTA to PBS.

4. Pass this solution through a 0.22 μm PES and vacuum filter. Aliquot the solution into working vials if required and label appropriately. The diluted stock of 0.5mM EDTA can be stored at ambient temperature for up to 6 months.

5. Aspirate differentiated colonies from the culture vessel with a Pasteur pipette.

6. Remove spent media from each vessel and wash with PBS; 1 mL/well of 6-well plate or 5 mL/100 mm dish.

7. Add appropriate volume of 0.5mM EDTA to each culture vessel and incubate immediately at 37ºC ± 0.5ºC/5% ± 0.5% CO_2 for 2-5 minutes. Use a laboratory timer to ensure accuracy of incubation time.

8. Remove the 0.5mM EDTA from the vessel. Do this tilting the vessel forward slightly to collect the EDTA in the bottom edge of the vessel.

9. Immediately add an appropriate volume of mTeSR1 to neutralize the EDTA: 1 mL/well of 6-well plate or 6 mL/100mm dish. Three gentle aspirations will dislodge cell clusters without dislodging any differentiated cells.
   NOTE: do not over aspirate the cells as this will result in single cells rather than cell clusters.

10. Seed the cells at an appropriate ratio (1:4 to 1:6) into a new matrigel-coated vessel (see SOP iPSC PT02-2v1).

11. To ensure even distribution of cell clusters, gently disperse the clusters by carefully moving the vessel side to side, back and forth several times before placing it in an incubator maintained at 37ºC ± 0.5ºC/5% ± 0.5% CO_2.

12. After 48hs, change culture medium daily until the new colonies will be ready for a new passage (usually every 6-7 days).

NOTE: routine testing for mycoplasma contamination must be carried out during this culture period.

PROTECTION MESSURES FOR USING THIS TECHNIQUE

- Take care when handling cutting material and needles during and after use, as well as throughout its clean-up and elimination procedures.

- Sharp objects (needles, syringes and other sharp instruments) must be deposited in appropriate containers with safety lids to prevent loss during transport. These containers are placed near the workplace and should not be overfilled. These objects must be disposed of as provided for specific medical waste or type III.

- Avoid wounds and scratches in handling of parts and accessories of instruments that can be sharp and in the access to difficult areas.

- Use of biosafety hoods in combination with additional personal protective equipment (Biological Safety Cabinets Class II will be used).

- Washing hands after handling biological material and before leaving the laboratory.

PROTECTION EYES / FACE

Use of biosafety hoods. If it is not possible, safety goggles should be used in those cases where, by the nature of the procedure performed, splashes affecting the mucous membranes of eyes are expected.

Face shields should be used in situations of risk where eye protection should be extended to the face.

The use of surgical masks could be considered sufficient against biological risks coming from splashes. However, these masks are not considered personal protective equipment for the respiratory system.

SKIN PROTECTION

The continuous use of gloves is mandatory in all operations.

Hands and arms are normally the parts of the body that more frequently come into contact with sharp objects and splashing. Gloves and sleeves garments are ideal for protecting hands and arms.

Gloves to protect against biological agents must be waterproof, flexible and with great sensitiveness to enable use in all types of work. When it is required, they should be sterile.

WHAT TO DO IN CASE OF EMERGENCY: BIOLOGICAL AGENT (leak, spillage, etc.)

In case of a leak, spillage and accident, such as inoculation, cut and pricks to the skin, inform immediately the person in charge of Emergencies, your direct head (Head of Department, Platform or Laboratory), and the person in charge for the Safety at the workplace.

FIRST AID INSTRUCTIONS

After inoculations, cuts or pricks to the skin: A small hemorrhage has to be provoked and the wound has to be washed with water and neutral soap, do not rub, and add some iodized Povidona.

After sprinklings on the skin: Wash the affected area with abundant cold water and neutral soap, without rubbing, for 10 minutes.

After sprinklings in the eyes: Wash the eyes with water in the special basin for the eyes and keep the eyelids open, for 20 minutes.

In all cases and after the first cure, the biological agent involved in the accident and the origin of the leak has to be identified, inform the person in charge for the Safety at the workplace and go to see the doctor of the insurance company for work-related accidents (Mutua).

Biological spillage

Disinfect the area contaminated with a 10 % dilution of lye.

If it is necessary, disinfect the area with antifog fluid.

If the paper forms of the laboratory or other manuscript or printed paper are contaminated, the information shall be copied in another document and the original has to be thrown in the container for contaminated waste.

Emission of potentially infectious aerosols (out of a camera of biological safety)

Everyone should evacuate the affected area immediately; those exposed to the emission should be sent immediately to receive medical attention. Nobody will be able to enter the area during a specified time, so that the aerosols could go out and the heaviest particles

settle. If the laboratory is not fitted with a central air evacuation system, the access will be delayed.

Signs will be placed indicating that entry is forbidden. After the appropriate time, the decontamination has to be done under the supervision of the person in charge of the Laboratory. For this it will be necessary to use protective clothes and suitable breathing equipment.