SOP iPSC PT04-2v2	Title: **Culture and Maintenance of Pluripotent Stem Cells on matrigel and mTeSR1 culture media**

Prepared by: Laia Miquel	Revised by: Begoña Aran	Approved by: Anna Veiga
Date: 20/04/15	Date: 17/04/18	Date: 30/04/18

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 PT04-1v2 Cryopreservation and Recovery Upon thaw of PSCs on matrigel and mTeSR1 culture media
SOP iPSC PT02-1v2 Preparation of mTeSR1 Media for the maintenance of PSC
SOP iPSC PT02-2v2 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)
- 10 mm 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 PT02-1v2)
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 characterization).

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 (50µl EDTA/ 50ml PBS)
4. Pass this solution through a 0.22 m PES 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 2-3 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, gently wash the mTeSR1 over the vessel with a P1000 pipette to dislodge the cells from the plastic. Do not aspirate more than 3 times to avoid breaking the cell clumps into single cells.
10. Pass the clumps to a 50 mL Falcon tube.
11. Seed the cells at an appropriate ratio (1:4 to 1:6) into a new matrigel-coated vessel (see SOP iPSC PT02-2v2). The final volume must be 2mL/well of 6w plate or 10mL/10mm plate.
12. 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.
13. 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.