Standard Operating Procedure
for
Culturing hESCs in conditioned medium or in chemically defined medium (mTeSR)

1.0 PURPOSE

This procedure describes the method of culturing hESCs on Matrigel-coated dishes using conditioned medium[1] or chemical defined medium (mTeSR, StemCell Technologies, Vancouver, BC).

2.0 SCOPE (Should include which Cores this SOP applies to)

This procedure applies to all personnel who need to culture hESCs on Matrigel-coated dishes.

3.0 PROCEDURE

3.1 Prepare Matrigel-coated dishes

1. Thaw one Matrigel aliquot in a 4°C refrigerator from -20°C freezer overnight. Also place a box of 20-200 µL tips in the 4°C refrigerator.
2. To coat a 6-cm dish, add 2 ml of cold, sterile DMEM/F12 to a 15 mL conical tube.
3. Take the box of 20-200 µL tips from the refrigerator and use pipette to take 30 µl Matrigel from the Matrigel aliquot into 15 mL conical tube.
4. Use a 5-mL pipet to mix Matrigel solution homogeneously.
5. Transfer 2 ml of Matrigel solution onto 6-cm dish and incubate at 37°C for 1 hour.

3.2 Acquire hESC from ready-to-passage dish

1. Remove a 6-cm hESC dish from incubator and aspirate the spent conditioned medium (CM) or chemically defined medium (e.g., mTeSR).
2. Wash once with 2 ml PBS. Then add 2 ml of pre-warmed Dispase solution[2] to 6-cm dish. Incubate at 37°C for 5-10 min. Separation of colonies from the dish can be confirmed by checking highlighted or folded-back edges of the colonies under microscope.
3. Aspirate the Dispase solution carefully. The hESC colonies should still attach to the dish.
4. Add 2 ml of CM or mTeSR medium and scrape the colonies off the dish with a 5-mL glass pipette while keep pipetting the cell solution up and down.
5. Collect the 2 ml cell solution into a 15 mL conical tube. If there are still colonies left on the old dish, add another 1ml fresh (CM) or mTeSR medium to collect the colonies into the 15 mL conical tube.
6. Centrifuge hESC cell solution at 200xg for 5 minutes.
7. Aspirate the supernatant from the hESC pellet and re-suspend the pellet with 4 mL CM or mTeSR medium or desired volume which depends on split ratio.

3.3 Plate hESC onto Matrigel-coated dish

1. Gently pipet cell solution in conical tube up and down to break up pellet into small colonies.
2. Take Matrigel-coated dish from the incubator prepared in 3.1 and aspirate DMEM/F12 solution.
3. Wash Matrigel-coated dish with 2 ml of PBS and add 3 ml of CM or mTeSR medium.
4. Take 1 ml hESC cell solution from Step 7 and plate to Matrigel-coated dish. To ensure even distribution of hESC colonies, add the cell solution drop-wise in a circular motion.
5. Move the dish in back-and-forth and side-to-side motions to further ensure even distribution.
6. Label the new hESC dish with cell line name, passage number and other necessary information.
7. Return the dish to the incubator and change 4 ml of medium daily for 6-cm dish.

[1] Preparation of hESC CM

- Plate 4.2 million of MEFs in a T75 flask with 15 ml MEF medium.
- Deactivate cells by treating with 150 µl of the 1 mg/mL mitomycin C solution.
- Incubate for 1.5 hr, aspirate MEF medium.
- Wash once with PBS, add 8 ml MEF medium and incubate for another 1.5 hrs.
- Aspirate MEF medium.
- Add 37 ml of **hESC medium without bFGF** and incubate for 1 day,
- Collect hESC medium from T75 flash and store hESC CM at -20^{o}C or -80^{o}C.
- For use, thaw and supplement with 4 ng/mL bFGF.

[2]Dispase Solution, 2mg/ml:

Ingredients	**Amount**
Dispase	80 mg
DMEM/F12 basal media	40 ml

Filter with 0.22 µm syringe filter and keep sterile.

E. Tzanakakis/J. Wu 8/27/12