Standard Operating Procedure
for
PREPARING MEF CULTURE DISHES

1.0 PURPOSE

This procedure describes how to prepare MEF culture dishes.

2.0 SCOPE

This procedure applies to all personnel who wish to prepare MEF culture dishes.

3.0 PROCEDURE

1. Add 2 ml 0.1% gelatin solution to each well of 6-well plates or 6 ml to 10-cm dishes. Incubate 1-2 hour at 37°C or overnight at room temperature.
2. Thaw feeder cells in a 37°C water bath. When only a small piece of ice remains, remove the cells from water bath. Spray the outside of the vial with 70% ethanol before move it into the hood.
3. Add the thawed cells into a 15-mL tube containing 5 ml pre-warmed MEF medium medium.
4. Rinse the vial with 1 mL pre-warmed medium. Transfer the medium to the same 15-mL tube. Gently the cells mix.
5. Centrifuge the cells at 200 × *g* for 5 minutes.
6. Aspirate the supernatant and resuspend the cells.
7. Aspirate the gelatin solution from the gelatin-coated culture vessel and wash the vessels once with PBS.
8. Add a suitable volume of MEF medium and feeder cells suspension into each culture vessel.
   Note: The recommended density of feeder cells is 2×10^4 cells/cm^2.
9. Distribute the cells by moving the culture vessels back-and-forth and side-to-side.
10. Incubate the cells in a cell incubator overnight and use the feeder culture vessels with 3–4 days.

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