Culturing a primitive neuroectoderma tumor cell line, RS-2 in RD6F

REQUIRED MATERIALS

Required Equipment

Sterile biosafety cabinet

Microscope

37°C/ 5% CO 2 Incubator

Pipet-Aid

Liquid Waste disposal system for aspiration

Reagents

- ◆RD medium**
- ◆ 6F***
- ◆ 1% Soybean trypsin inhibitor dissolved in MCDB 153 (or dilute RD medium 50 folds by adding PBS)
- ◆ 0.05% trypsin / 0.04% EDTA 4Na in PBS
- ◆ PBS(-)

Required Supplies

- ◆ 5ml sterile serological pipets
- ◆ 10ml sterile serological pipets
- ◆ 15ml centrifuge tubes
- ◆ 50ml centrifuge tubes
- ◆ Baked Pasteur pipets
- ◆ 25cm^2 Flask *

* **Corning flask is recommended**. If others are used for the culture, it is recommended to be coated bovine fibronectin (2 μ g/cm^2). Coated flask which can be kept at 4 °C should be used within 3 days.

** **RD medium** is a 1:1 mixture of RPMI 1640 medium and Dulbecco's modified Eagle' me-dium supplemented with sodium pyruvate (2mM), sodium bicarbonate (2.2g/L) and HEPES (15mM). pH 7.4. It is available to custom-order the medium by the Cell Science & Technol-ogy Institute (CSTI, Sendai, Japan http://www.cstimedia.com/english.html).

*** **6F** consists of bovine insulin (10 μ g/ml), human transferrin (5 μ g/ml), fatty acid-free bo-vine serum albumin (50 μ g/ml), 2-mercaptoethanol (10 μ M), 2-ethanolamine (10 μ M) and sodium selenite (10nM).

REAGENT PREPARATION

1. 6F are added to RD medium. (RD6F: use within one week.)
2. Soybean trypsin inhibitor (1%) is diluted 10-folds by adding MCDB 153 (or by RD medium 50 folds by adding PBS) and then sterilized by 0.22 mm filter.

PASSAGE PROCEDURE

1. Remove the culture medium.
2. Add 5ml PBS(-) gently.
3. Remove PBS.
4. Treat the cells with 0.5ml of 0.05% trypsin / 0.04% EDTA 4Na for 1~2 minutes at the room temperature.
5. Remove the trypsin /EDTA gently.
6. Add collect the cells in 9ml of PBS into 15ml tube and pipette them.
7. Add 1ml of 0.1% soybean trypsin inhibitor.
8. Centrifuge the cells at 1200rpm for 3 min at 4 ^{o}C.
9. Remove the supernatant.
10. Tap the tube to deform the cell pellets.
11. Add 5ml PBS into the tube and pipette well.
12. Add 5ml RD medium into the cell suspension solution slowly and pipette gently.
13. Centrifuge the cells at 1200rpm for 3 min at 4 ^{o}C.
14. Remove the supernatant.
15. Tap the tube to deform the cell pellets.
16. Add RD6F into the tube and pipette well.
17. Seed the cells into 25cm^2 flask (1:3~6 split, at the cell density of 1~2 x10^4 cells/cm^2).
18. Culture the cells in a humidified atmosphere of 5% CO^2 at 37^{o}C.
19. Change the medium every 2 days.

Note: Sometimes, the cells aggregate each other and make spheroid. In that case, collect the cells into 15ml tube and treat the cells with 0.01% EDTA 4Na /PBS.