Culutre cells
Thawing cells
Material
- Cells
- Medium : DMEM + 10% FBS
Procedure
1. Transfer the cell in liquid nitrogen to a freezer at -80°C for 20 minutes to remove nitrogen.
2. Measure 8ml of medium into a centrifuge tube with a pipette.
3. Take out the Vero cell from the freezer, and half-thaw it in a 37°C water bath.
4. Take about 1ml of medium with a pipette and suspend the cell.
5. Put the suspension into a centrifuge tube.
6. Centrifuge at 800-1000 rpm for 5 min.
7. Discard the supernatant from the centrifuge tube using a suction pump (leave about 1 cm of medium in the centrifuge tube).
8. Measure out 3ml of medium with a pipette and suspend the remaining cell & medium.
9. Spread the suspension into a Φ60 dish and shake the dish back and forth and side to side to avoid cell bias.
10. Place in incubator.

Cell freezing
Material
- Cells
- PBS (-)
- 0.25% trypsin
- DMEM + 10% FBS (:Medium)
- Medium + 20% DMSO
Procedure
1. Discard medium with a suction pump.
2. Wash the dish with PBS three times to remove medium.
3. Add trypsin and spread it throughout
4. 0.5mL with φ100
5. Incubate at 37°C for 2min
6. Confirm cell detachment under a microscope
7. Trypsin inactivation and centrifugation
8. Wash the entire bottom of the dish several times to remove cells.
9. Keep the pipette tip perpendicular to the bottom of the dish.
10. Centrifuge the cells in a centrifuge tube at 800 rpm for 5 min.
11. Discard the supernatant and resuspend the pellet in medium.
12. Add an equal volume of chilled medium + 20% DMSO to the medium used in the step before.
13. Dispense into cryo tubes
14. Store overnight at -80°C in an appropriate container.

Medium change
Material
- Cells

- Medium : DMEM + 10% FBS

Procedure

1. Warm medium (DMEM + 10% FBS) in a 37°C water bath.
2. Remove the dish containing the Vero cell from the incubator.
3. Sterilize the tip of the suction pump and attach a yellow tip while sucking up.
4. Discard medium in the dish, taking care to minimize the contact area between the tip and the side of the dish.
5. Measure out new medium from the container with a pipette equipped with a pipette aid.
6. Pour a small amount of medium into the dish.

Immediately cover, photograph, and place in incubator

Passege

Material

- PBS(-)
- 0.25% trypsin
- DMEM + 10% FBS

Procudure

1. Thaw trypsin stored at -40°C overnight at -4°C
2. Keep warm at 37°C WB
3. Suck medium in the dish with a suction pump
4. Wash out the remaining medium in the dish with PBS and suction.
5. Add trypsin to the dish and tilt it up, down, left and right about 10 times to spread the trypsin throughout the dish.
6. Incubate at 37°C for 3 min.
7. Confirm under a microscope that Vero has left the dish.
8. Inactivate trypsin by adding Medium to the dish
9. Transfer 8 to a centrifuge tube and centrifuge at 800 rpm for 5 min.
10. If the volume of Medium is too large, add a certain amount of Medium to the dish in advance.

φ100: Medium 9mL

In the above case, add 8mL in advance.

12. Discard the supernatant in the centrifuge tube and dissolve the pellet in Medium.
13. Spread the pellet into the dish, and tilt it up and down, left and right, about 10 times so that the pellets are spread over the whole dish.

