September 15, 2023, 16:10-18:20

Introduction of cell culture and passaging

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Lecture (15:30-16:00)
Basic Lecture and Practice Building, 2nd floor

Split the attendees into two groups for the practice (16:10)
Group A (English): Asahina, Hirano
Group B (Japanese): Terado, Mori, Yamamoto

Practice (16:15-18:20)
Room 416, Central Research Laboratory

Materials
Cells
COS-7 fibroblast-like cell line (obtained from Dr. Akio Shimizu)
(transformed by polyoma virus SV40 derived from African green monkey kidney)

Culture medium
DMEM high glucose
10% fetal bovine serum
Glutamine
Sodium pyruvate
Sodium bicarbonate ($NaHCO_3$)
Antibiotics

Freezing medium (x2 concentration)
DMEM high glucose
20% fetal bovine serum (10% final concentration)
20% dimethyl sulfoxide (DMSO) (10% final concentration)

Trypsin-EDTA solution
2.5 g/L trypsin
1 mM EDTA

Trypan blue solution
0.05% trypan blue

Note. General plate area, cell number, and medium volume in different plates/dishes

	Area	Medium volume	Plate number	Confluent number
96-well plate	0.3 cm^2	0.1 ml	$6.0x10^2$ - $3.0x10^3$	$5.0x10^4$
48-well plate	0.7 cm^2	0.2 ml	$1.4x10^3$ - $7.0x10^3$	$1.3x10^5$
24-well plate	2 cm^2	0.5 ml	$4.0x10^3$ - $2.0x10^4$	$2.5x10^5$
12-well plate	4 cm^2	1 ml	$8.0x10^3$ - $4.0x10^4$	$5.0x10^5$
6-well plate	10 cm^2	2 ml	$2.0x10^4$ - $1.0x10^5$	$1.2x10^6$
35 mm dish	10 cm^2	2 ml	$2.0x10^4$ - $1.0x10^5$	$1.2x10^6$
60 mm dish	20 cm^2	5 ml	$4.0x10^4$ - $2.0x10^5$	$2.5x10^6$
100 mm dish	60 cm^2	10 ml	$1.2x10^5$ - $6.0x10^5$	$7.5x10^6$

Procedures

1. Passaging

1. Take the 60 mm culture dish from the CO_2 incubator
2. Observe cells under the microscope
3. Move the dish into a culture hood (clean bench)
4. Aspirate the culture medium using a Pasteur pipette and vacuum pump
5. Add 2 ml of PBS to the dish
6. Aspirate the PBS
7. Add 1 ml of a Trypsin-EDTA solution to the dish
8. Incubate the dish in the CO_2 incubator for 3 min
9. Check whether cells detach from the dish
10. Add 2 ml of the culture medium
11. Dissociate cells by pipetting 10 times
12. Take 10 μl from the cell suspension
13. Add 10 μl of trypan blue solution to cells and mix well
14. Apply 10 μl of cell suspension to a hemocytometer
15. Count the cell number
    The average cell number in a square x 10^4 cells / ml
16. Meanwhile, transfer cells to a 15 ml tube
17. Centrifuge the tube at 3,000 rpm for 3 min
18. Suspend the cell pellet in 1 ml of the medium
19. Plate 1 x 10^5 cells in 5 ml medium into a new 6 cm dish

2. Cryo-preservation

1. Transfer 500 μl of the cell suspension prepared in (1-18) into a cryotube
2. Add 500 μl of a freezing medium
3. Mix the cells gently
4. Put the tube into a freezing container
5. Keep the freezing container in -80°C freezer for 24 hrs
6. Transfer the freezing tube into the liquid nitrogen tank next day

3. Plating cells from freezing stock

1. Aliquot 10 ml of culture medium in a new 15 ml tube
2. Incubate the tube in the water bath at 37°C
3. Take a cryotube from the liquid nitrogen tank
4. Thaw the cryotube in the water bath at 37°C
5. Transfer cells from the cryotube to the pre-incubated medium
6. Centrifuge the 15 ml tube at 3,000 rpm for 3 min
7. Suspend the cell pellet in 1 ml of the culture medium
8. Count the cell number
9. Plate 1x10^5 cells in a new 6 cm dish