

Cell Culture and Passaging **(Practice in English/Japanese)**

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細胞培養の方法について概説し、実際に細胞を取り扱った実習を行う。培養皿上で培養した細胞を観察後、トリプシン-EDTA を使って細胞を剥離する方法を学び、剥離した細胞の数を血球計算盤でカウントし、新しい培養皿に播種する一連の継代操作を習得する。

We will explain how to culture a cell line on a plastic dish in a sterile condition. In the following hands-on training, we will show you how to collect cells growing on the dish using a trypsin-EDTA solution, count the cell number with a cell counter, and passage them to a new dish.

Sep 11, 2026
17:00-18:20

Introduction of cell culture and passaging

細胞培養の基礎と細胞の継代方法

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Cell culture

Adherent cells

Plastic dish (plate)

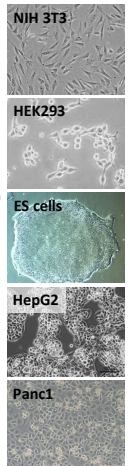


Cell lines

NIH 3T3 (mouse fibroblasts)
HEK 293 (human embryonic kidney cells)
COS-7 (monkey fibroblasts)
CHO (hamster ovary cells)
Embryonic stem cells, etc...

Cancer cell lines

HepG2 (human hepatoma)
Panc1 (human pancreatic cancer)
etc...



Culture medium

Dulbecco's Modified Eagle Medium (DMEM)

Buy either powder or liquid (filtered)

Components

D-Glucose (1,000 mg/ml low or 4,500 mg/ml high)
L-Glutamine ← Unstable, need to be added
Sodium pyruvate
NaHCO₃ ← Makes pH stable
HEPES ← Makes pH stable
Phenol red ← pH indicator

Inorganic components

CaCl₂, KCl, MgSO₄, NaCl, NaH₂PO₄, Fe(NO₃)₃

Amino acids

L-Arginine, L-Cystine, Glycine, etc...

Vitamins

Folic acid, Inositol, etc...

There are different media available (MEM, RPMI 1640, IMDM, etc...).
Select the best medium for your cell type



Sterilization of liquid by filtration

Culture media or bio reagents

0.2 µm filter

(cannot exclude mycoplasma)

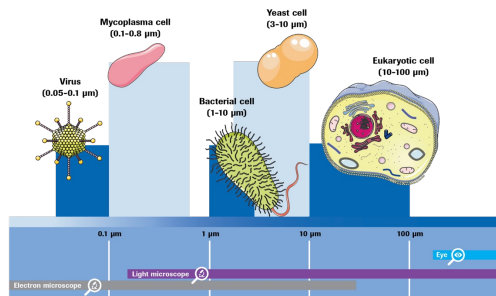


Large units for making culture medium (100-1,000 ml)

Small unit for making reagents (1-10 ml)



Mycoplasma



Mycoplasma cannot be removed by filtration using a 0.2 µm filter.

Antibiotics

Anti-bacteria

Penicillin G: Inhibition of the bacterial cell wall synthesis

Streptomycin: Inhibition of protein synthesis

Gentamycin: Inhibition of protein synthesis

Anti-fungal

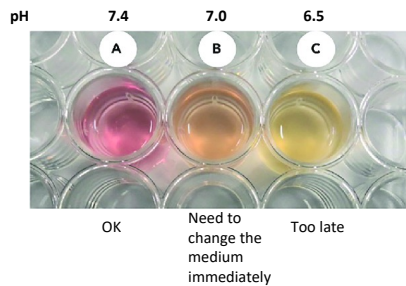
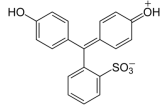
Amphotericin B: Suppression of the growth of fungi



These antibiotics cannot prevent the contamination of viruses, mycoplasma, and yeast.



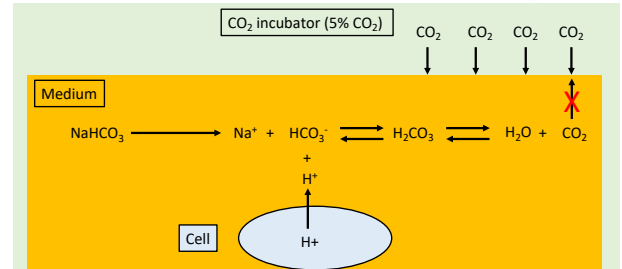
Phenol red



CO₂ incubator

1. 37 °C temperature
2. 90-95% moisture (use water chamber)
3. 5% CO₂ for pH stabilization

Available on the 4th floor of the Central Research Laboratory



Serum for cell growth

Sera contain many factors that help cell growth (proteins, lipids, other components)

Fetal bovine serum (2-10%)

Horse serum (5%)

Heat-inactivation of fetal bovine serum (FBS)

Inactivate the component system containing in FBS by incubation at 56 °C for 30 min



Cell culture dish/plate/flask

Adherent cells

Plastic dish, plate, flask
Glass plate (cover glass)

Dish coating

Poly-D-Lysine
Extracellular matrices (collagen, fibronectin, etc...)

Dish



Plate

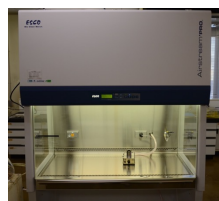


Flask

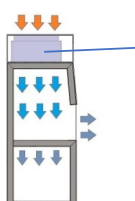


Cell culture hood

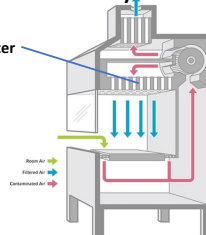
Available on the 4th floor of the Central Research Laboratory



Laminar flow hood



Biosafety cabinet



Aseptic techniques in cell culture

Cell culture hood

Gloves

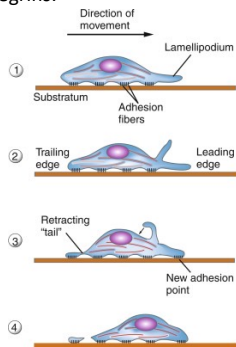
70% Alcohol



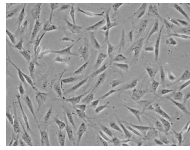
Cell adhesion on a culture dish

Epithelial cells and fibroblasts

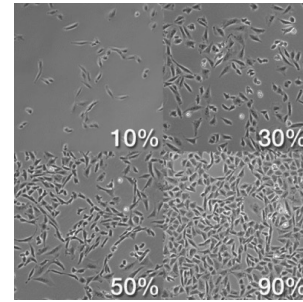
Adhere the extracellular matrix on the dish via proteins, such as integrins.



NIH 3T3



Confluency



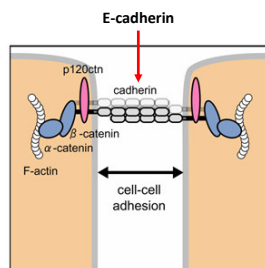
In general, cells grow well at a low confluency

Cells at a high confluency do not grow and respond to cytokines by their cell-cell contact inhibition.

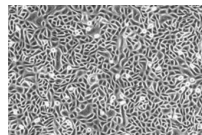
E-cadherin expressed on epithelial cells

Epithelial cells

Form epithelial colonies by E-cadherin, a Ca^{2+} -dependent cell-cell adhesion molecule



Keratinocytes



Passaging cells

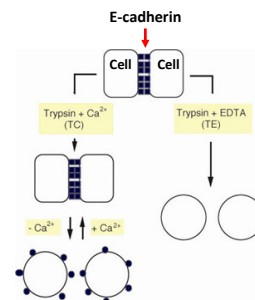
Use of Trypsin-EDTA for dissociation of cells from culture dishes

Trypsin (0.05-0.5%)

Digest proteins involving the cell attachment

EDTA

Chelate Ca^{2+} for dissociation of E-cadherin bindings



Passaging cells

General procedure

Check cells cultured on a dish under microscope

Aspirate the culture medium

Add PBS for washing

Aspirate the PBS

Add 2 ml of a Trypsin-EDTA solution to a 10 cm dish

Incubate at 37°C for 1-5 min (until cells detach from the dish)

Add 3 ml culture medium containing FBS

Dissociate cells by repeating pipetting 3-10 times gently

Transfer cell suspension to a 15 ml tube

Centrifuge at 3,000 rpm for 3 min

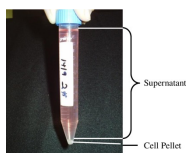
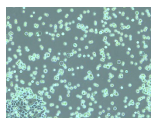
Aspirate the culture medium

Suspend the cell pellet in 1 ml medium

Take 10 μl of cell suspension

Add 10 μl of trypan blue to detect dead cells

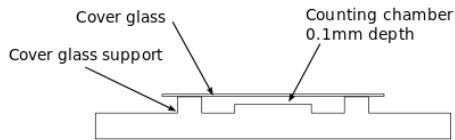
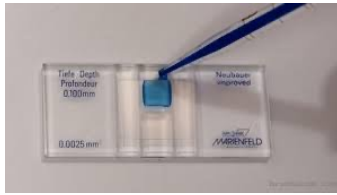
Count the cell number



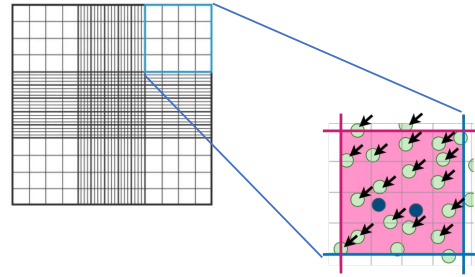
Trypan blue dye



How to count the cell number using a hemocytometer?



Under the microscope, you will see these squares



Arrows indicate live cells.

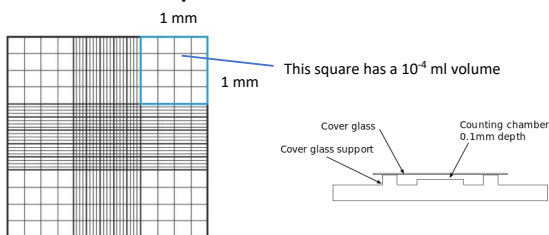
You need to count cells on the 2 border lines (red lines in this figure).

Do not count cells on the all 4 border lines (red and blue lines).

In this picture, there are 17 live cells (arrows) and 2 dead cells (blue).

The viability is $17/(17+2) \times 100 = 89.5\%$.

One square has a 10^{-4} ml volume



One square of this plate: 1 mm x 1 mm x 0.1 mm

1 ml volume: 10 mm x 10 mm x 10 mm

10-fold x 10-fold x 100-fold = 10,000-fold

Cell concentration = average number x 10^4 cells/ml
(keep this number in mind)

Count cell number in each of 4 squares

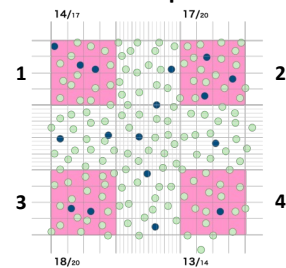
Example

Cell suspension in 5 ml

Take 10 ul of cells

Add 10 ul of trypan blue

Use 10 ul for cell counting



Total cell number

$(17+20+20+14) / 4 \text{ squares} \times 2 \text{ (trypan blue dilution)} = 35.5 \text{ cells}$

$35.5 \times 10^4 = 3.55 \times 10^5 \text{ cells/ml (cell concentration)}$

$3.55 \times 10^5 \text{ cells/ml} \times 5 \text{ ml} = 1.78 \times 10^6 \text{ cells (total number)}$

Viability

Live cells: $(14+17+18+13) / 4 \times 2 = 31 \text{ cells}$

$31 \times 10^4 \text{ cells/ml} \times 5 \text{ ml} = 1.55 \times 10^6 \text{ cells}$

$1.55 \times 10^6 \text{ cells} / 1.78 \times 10^6 \text{ cells} \times 100 = 87.1\%$

How to preserve cultured cells?

Collect cells from the dish with a trypsin-EDTA solution

Count cell numbers

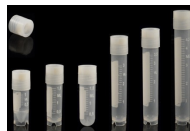
Suspend 10^6 - 10^7 cells in 1-2 ml of the stock solution

(Stock solution: 10% dimethyl sulfoxide, 20% FBS in DMEM)

Aliquot cell suspension in a cryovial

Put the vial in a freezing container

Freeze cells slowly in -80°C freezer overnight



Add isopropanol

Storage of cells

Liquid Nitrogen Dewar

Liquid nitrogen (-196°C)

Liquid nitrogen increases its volume

800-fold when it becomes gas.

Never store liquid nitrogen in closed containers or tubes.

If liquid nitrogen is present in the closed tube, the tube will explode during thawing.

Liquid nitrogen Dewar

Store cell vials in liquid nitrogen

Need to add liquid nitrogen periodically

Mycoplasma might be contaminated from tubes to tubes via liquid nitrogen



Storage of cells



Air liquid nitrogen tank

Store cell vials in the very cool air generated by liquid nitrogen

Available on the 4th floor of the Central research laboratory

Need to add liquid nitrogen periodically

Free from mycoplasma contamination

Next

Observe cultured cells under the microscope

Remove cells using trypsin-EDTA

Count cell numbers

Passage to a new dish

Make a freezing stock

Take a frozen cell vial from a liquid nitrogen tank

Plate cells to a new dish

Introduction of cell culture and passaging

Kinji Asahina, Futoshi Toyoda, Takefumi Yamamoto, Yasuhiro Mori, Tokio Terado,
Kumi Okamoto, Akari Uno (Central Research Laboratory)

Lecture (17:00-17:20)

Room 416, Central Research Laboratory

Practice (17:20-18:20)

Room 416, Central Research Laboratory

Split the attendees into two groups for the practice

Group A (English): Asahina

Group B (Japanese): Okamoto, Toyoda

Materials

Cells

CHO (Chinese hamster ovary) cells

Culture medium

DMEM/Ham's F-12

10% fetal bovine serum

Glutamine

Sodium pyruvate

Sodium bicarbonate (NaHCO_3)

Phenol red

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	6.0×10^2 - 3.0×10^3	5.0×10^4
48-well plate	0.7 cm ²	0.2 ml	1.4×10^3 - 7.0×10^3	1.3×10^5
24-well plate	2 cm ²	0.5 ml	4.0×10^3 - 2.0×10^4	2.5×10^5
12-well plate	4 cm ²	1 ml	8.0×10^3 - 4.0×10^4	5.0×10^5
6-well plate	10 cm ²	2 ml	2.0×10^4 - 1.0×10^5	1.2×10^6
35 mm dish	10 cm ²	2 ml	2.0×10^4 - 1.0×10^5	1.2×10^6
60 mm dish	20 cm ²	5 ml	4.0×10^4 - 2.0×10^5	2.5×10^6
100 mm dish	60 cm ²	10 ml	1.2×10^5 - 6.0×10^5	7.5×10^6

Procedures

1. Passaging

1. Take the 60 mm culture dish from the CO₂ incubator
2. Observe cells under the microscope
3. Move the dish into the culture hood
4. Aspirate the culture medium using a Pasteur pipette
5. Add 2 ml of PBS to the dish
6. Aspirate PBS
7. Add 1 ml of Trypsin-EDTA solution to the dish
8. Incubate the dish in the CO₂ incubator for 3 min
9. Check whether cells detach from the dish
10. Add 2 ml of the culture medium
11. Dissociate cells by gentle pipetting around 10 times
12. Aliquot 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 $\times 10^4$ 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 1×10^5 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 1×10^5 cells in a new 6 cm dish