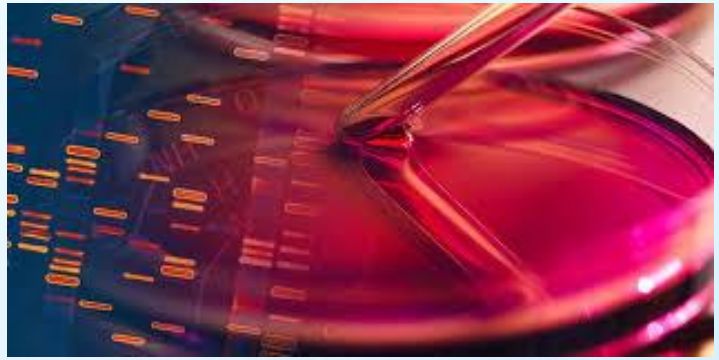
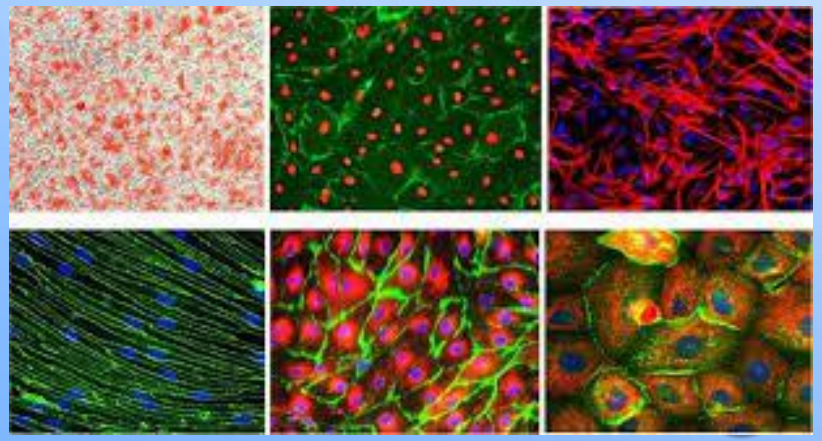


TITLE:

CELL CULTURE TECHNIQUES

Presented By: Hoorieh Shapourian



OUTLINES

- An overview on cell culture
- Why is cell culture used for?
- Culture media and required equipment
- Culturing of adherent and suspend cells
- Subculture of cells
- Freezing cells for storage
- Working with cryopreserved cells
- THP-1 and its cell culture tips

AN OVERVIEW ON CELL CULTURE

- **Cell culture** is the process by which **prokaryotic, eukaryotic, animal or plant cells** are grown under controlled conditions, generally outside their natural environment.
- Cell culture was **first** successfully undertaken by **ross harrison** in 1907, He realized that the growth of **frog embryonic cells** would give rise to **nerve cells** in a medium of **clotted lymph**.

WHY IS CELL CULTURE USED FOR?

- **GOAL:** To study different aspects of plant and animal cells in the laboratory and in vitro
- **Checking the growth rate / the size of the cells / viability / cells metabolic process / cell activities**

- ***Applications:***
- **Model systems:** For studying **basic cell biology,** effects of drugs on cells
- **Toxicity testing:** Study the effects of **new drugs**
- **Cancer research:** Study the function of **various chemicals, virus & radiation** to convert normal cultured cells to cancerous cells or studying cancerous cell

CONTINUE...

- **Virology:** Cultivation of **virus for vaccine production**, also used to study **their infectious cycle**

- **Genetic engineering:** Production of **commercial proteins**, large scale production of viruses for use in vaccine production e.g. Polio, rabies, chicken pox, hepatitis B & measles

- **Gene therapy:** Cells having a functional gene can be replaced to cells which are having non-functional gene

CULTURE MEDIA AND REQUIRED EQUIPMENT

- ***CULTURE MEDIA:***
- Physiological fluid for cell survival and growth
- Choice of media **depends on the type of cell** being cultured
- **1. Basic culture medium:** ***RPMI, GMEM, EMEM,DMEM*** *etc.*
- **2. Rich culture medium: *ex-vivo***
- Media is supplemented with **antibiotics** viz. **Penicillin, streptomycin** etc.
- Prepared media is incubated at **4°C**

CONTINUE...

- ***REQUIRED EQUIPMENT:***
- Class II laminar flow
- Incubator: Co2 (5%), temperature (37°C), and humidity
- Fridge/freezer (-20, -70°C)
- Bain-marie
- Microscope (light and invert)
- Fridge centrifuge
- Nitrogen tank
- Cell culture plates and flasks with filter lid

CULTIVATION CONTAINERS AND NECESSARY INFORMATION

Dishes 	Surface area (cm^2)	Seeding density	Cells at confluency	Versene (ml of 0.05% EDTA). Approx. volume	Trypsin (ml of 0.05% trypsin, 0.53 mM EDTA).approx. volume	Growth medium (ml. approx. volume)
35mm	8.8	$0.3x10^6$	$1.2x10^6$	1	1	2
60mm	21.5	$0.8x10^6$	$3.2x10^6$	3	3	5
100mm	56.7	$2.2x10^6$	$8.8x10^6$	5	5	12
150mm	145	$5.0x10^6$	$20.0x10^6$	10	10	13

CONTINUE...

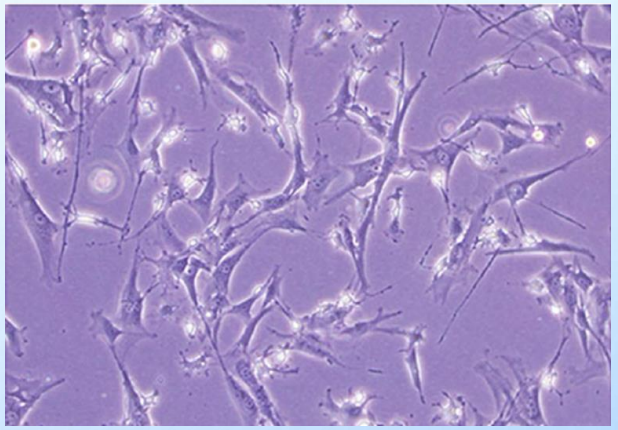
Plates 	Surface area (cm^2)	Seeding density	Cells at confluency	Versene (ml of 0.05% EDTA). Approx. volume	Trypsin (ml of 0.05% trypsin, 0.53 mM EDTA).approx. volume	Growth medium (ml. approx. volume)
6-well	9.6	$0.3x10^6$	$1.2x10^6$	1	1	1 to 3
12-well	3.5	$0.1x10^6$	$0.5x10^6$	0.4 to 1	0.4 to 1	1 to 2
24-well	1.9	$0.05x10^6$	$0.24x10^6$	0.2 to 0.3	0.2 to 0.3	0.5 to 1
48-well	1.1	$0.03x10^6$	$0.12x10^6$	0.1 to 0.2	0.1 to 0.2	0.2 to 0.4
96-well	0.32	$0.01x10^6$	$0.04x10^6$	0.05 to 0.1	0.05 to 0.1	0.1 to 0.2

CONTINUE...

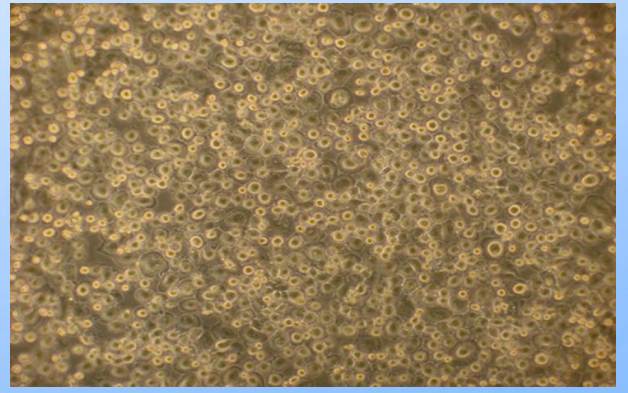
Flasks 	Surface area (cm^2)	Seeding density	Cells at confluency	Versene (ml of 0.05% EDTA). Approx. volume	Trypsin (ml of 0.05% trypsin, 0.53 mM EDTA).approx. volume	Growth medium (ml. approx. volume)
T-25	25	$0.7x10^6$	$2.8x10^6$	3	3	3-5
T-75	75	$2.1x10^6$	$8.4x10^6$	5	5	8-15
T-175	175	$4.9x10^6$	$23.3x10^6$	17	17	35-53
T-225	225	$6.3x10^6$	$30x10^6$	22	22	45-68

CULTURING OF ADHERENT AND SUSPEND CELLS

- **Adherent cells:** Tissue cells, different shapes: asteroid, spindle and …, stick to cell culture containers



- **Suspend cells:** HSC, PBMC, rounded, don't stick to cell culture containers



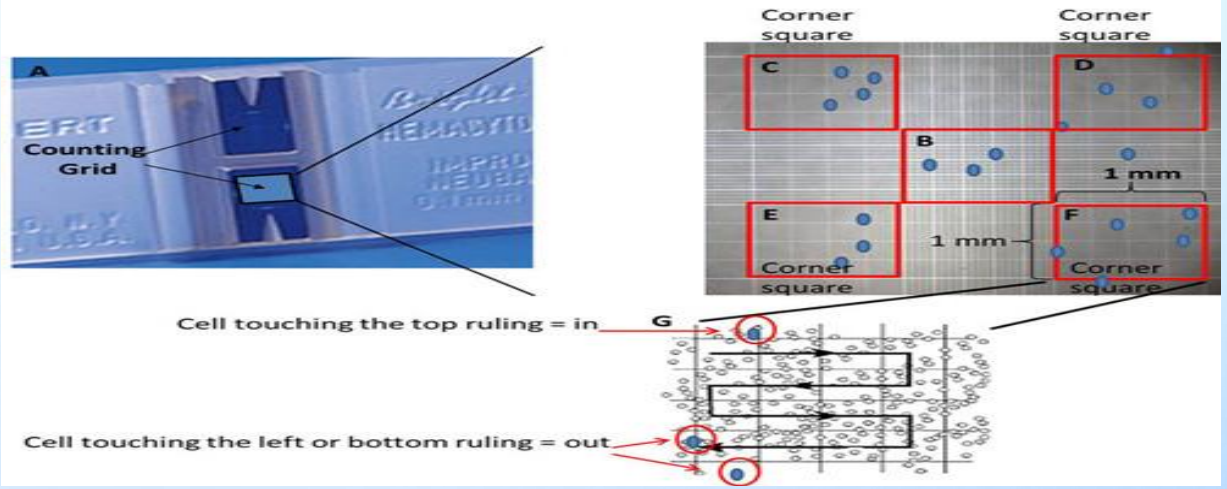
CULTURING OF ADHERENT CELLS

- 1. Removing the cells from the bottom of the culture medium plate or flask with **trypsin and EDTA**
- 2. Using the complete culture medium containing **10% FBS**
- 3. Centrifuge for **5 minutes at 900 RPM** or **3 minutes at 1200 RPM**
- ۴. **Drain** the supernatant and adding the necessary amount of **complete culture medium 10% FBS, 1 % pen/strep (1 ml) and resuspend cells**

CONTINUE...

- 5. Cell counting with neubauer slide:
- 10 λ cell + 10 λ Trypan blue

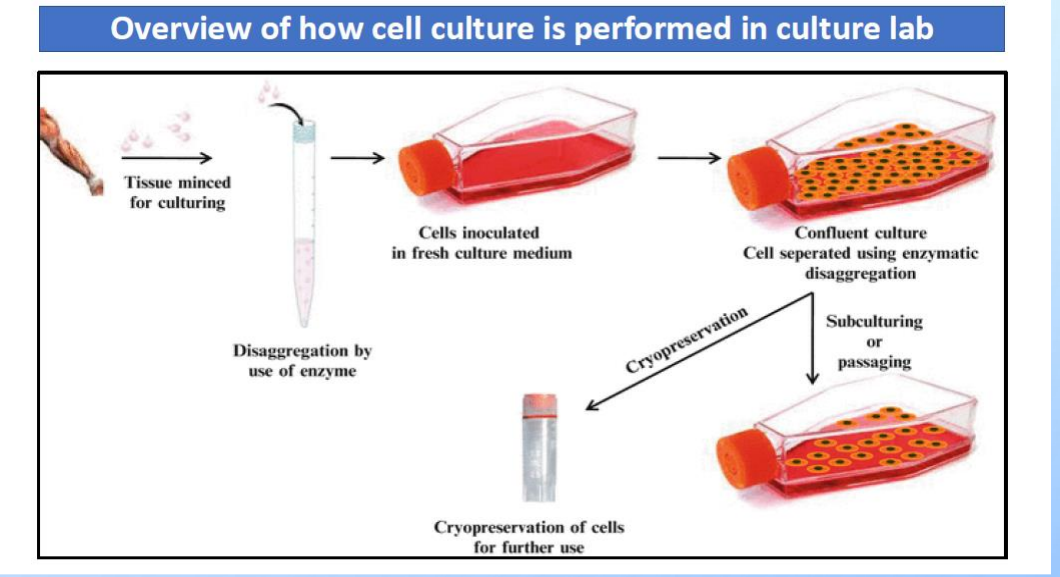
$$\text{Cell viability \%} = \frac{\text{Total cells - dead cells}}{\text{Total cells}} \text{X100}$$



- N = Average number of cells X 2 X The volume of the culture medium X 10^4
- 6. **Add** the culture medium based **on the required number of cells per ml**
- 7. Keeping cells in **appropriate ratio** of culture medium in **a flask or plate** in an **incubator**
- **The stages of *SUSPENDED CELLS CULTURE* are the same as the stages mentioned above, except for the first stage.**

SUBCULTURE OF CELLS

- **Subculturing or passaging** is either a new cell culture or a microbiological culture made by **transferring** some or all cells **from a previous culture** to **fresh growth medium.**
- Subculturing is used to **prolong the lifespan** and/or **increase the number of cells** or microorganisms in the culture.
- It's used when cells reach to **80-90% confluency** in flask/dishes/plates
- The steps are the same as the previous slides.



FREEZING CELLS FOR STORAGE

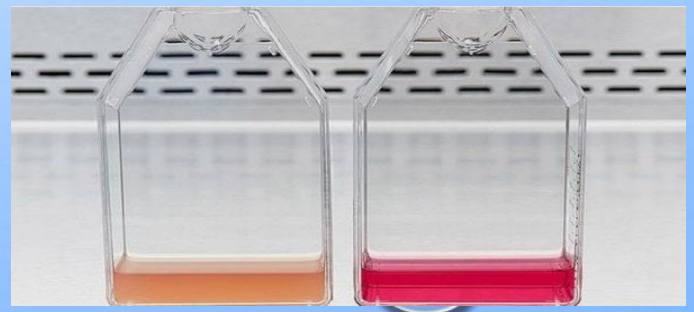
- **To save the cells** for future work and **prepare a cell bank** , we use **cryopreservation** of cells or **freezing** them. For this, we use tubes called **cryotubes**.
- **Steps:**
- **1.** In the cryotube, depending on the cell size, put at least **1 million cells** or more in **١ml of FBS** containing **10% Dimethyl sulfoxide (DMSO)** (100 λ DMSO + 900 λ FBS)
- **2.** Then keep cells at **-20°C** for **several hours to overnight**
- **3.** Then keep cells at **-70°C overnight**
- **4.** At the end keep cryotube in **a nitrogen tank** at **-196°C** for **several years**

WORKING WITH CRYOPRESERVED CELLS

- In order to reuse the cryopreserved cell, it is necessary to **defreeze** it. For this purpose:
- 1. Take the cryotube under the **laminar flow** and **loosen its screw** a little to out the **gas**.
- 2. Place the cryotube in a **37°C bain-marie** and put the cell in thermal shock (**1-2 min**).
- 3. Because DMSO is **toxic** to cells, immediately add **9** times the complete culture medium containing **10-20% FBS** to it in **15 ml falcon tube**.
- 4. Then **centrifuge** and **count** the cells and **dilute** them to the desired number in the culture medium.

CELL CULTURE TECHNIQUE TIPS

- The most important principle in working with cells is the **sterility** of the conditions
- **Clean, fresh lab coats and glove** should be worn at all times in the hood area and removed as soon as the area is left.
- Turn on **UV lamps** to disinfect the laminar flow.
- At beginning of work, Spray **ethanol** ۷۰% to the surface of laminar flow and every material and equipment use under it.
- Screen for infections **(mycoplasma)** at regular intervals.
- In antibiotic-free media, **signs of bacterial, yeast, and fungal infection** include **cloudiness** as well as **color** and **pH changes**.



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abm
Thawing Cells: Best Practices
Thaw cells quickly
DMSO
20°C
Don't over-thaw
(DMSO is toxic at room temperature)
Media
37°C
OR
20°C
Use pre-warmed media
Don't centrifuge at high speeds
Keep container cap out of the water

PROPOSAL

Evaluation of the relation between TIM-3/Galectin-9 axis and Glutamine metabolism in AML cell lines, HL-60 and THP-1

THP-1

- **THP-1** is a **monocyte** isolated from **peripheral blood** from an **acute monocytic leukemia patient.** This cell line can be used in **immune system disorder research, immunology research, and toxicology research.**
- **Product category:** human cells
- **Organism:** homo sapiens, human
- **Cell type and morphology:** monocyte
- **Tissue:** peripheral blood
- **Disease:** acute monocytic leukemia (AML-M5)

https://www.atcc.org/

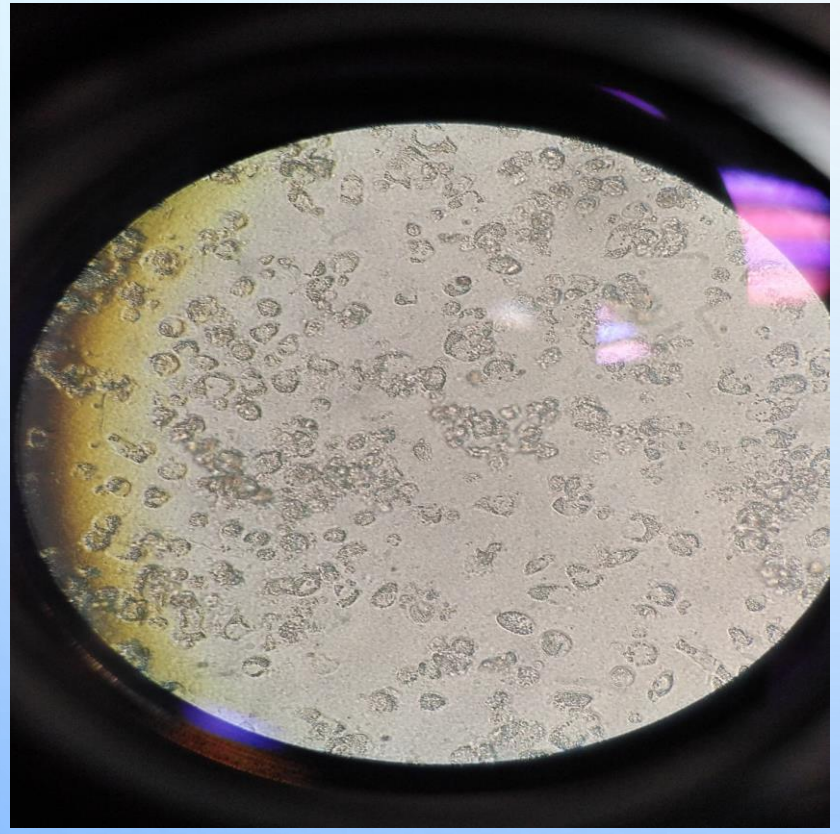
THP-1 CELL CULTURE TIPS

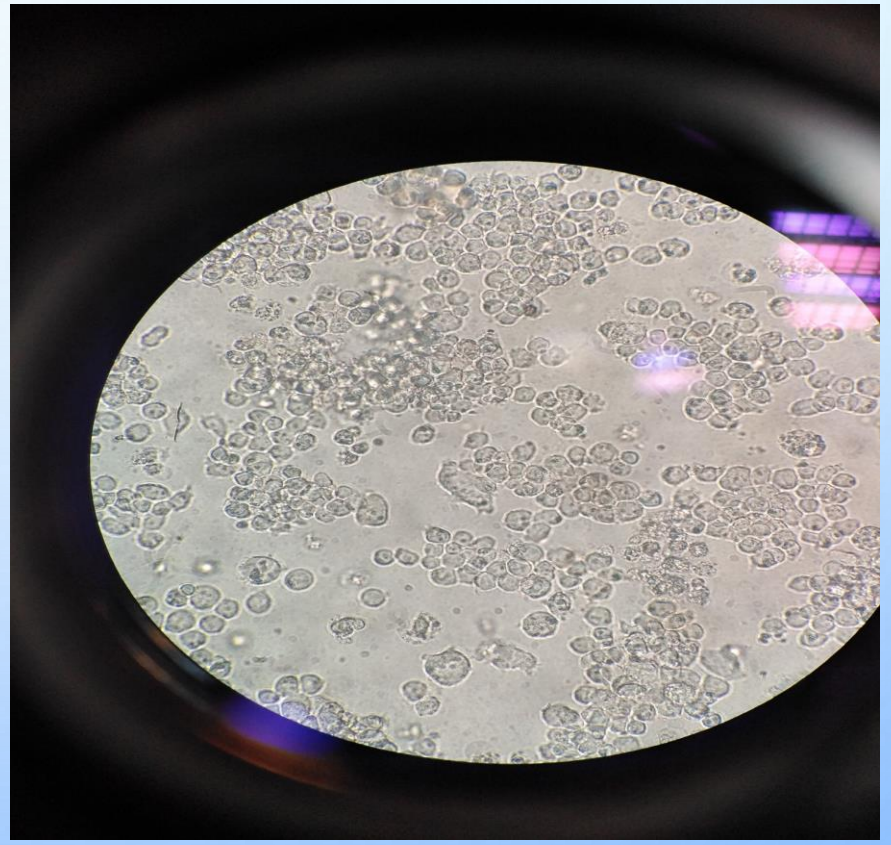
- The base medium for this cell line is **RPMI-1640 medium**.
- Prior to the addition of the vial contents, the culture vessel containing the **complete growth medium** be placed into the **incubator** for at least **15 minutes** to allow the medium to reach its **normal PH (7.0 to 7.6).**
- ATCC guideline said for complete growth medium, add **2mercaptoethanol (2me)** to a final concentration of **0.05 mM** and **FBS 10%** (but we used **2ME in 2mM** concentration and **15% FBS** and **1% L-Glu**).
- Cultures can be established by centrifugation with subsequent resuspension at **2-4 x 10^5** viable cells/ml.
- **Subculture** when cell concentration reaches **8 x 10^5** cells/ml.

CONTINUE...

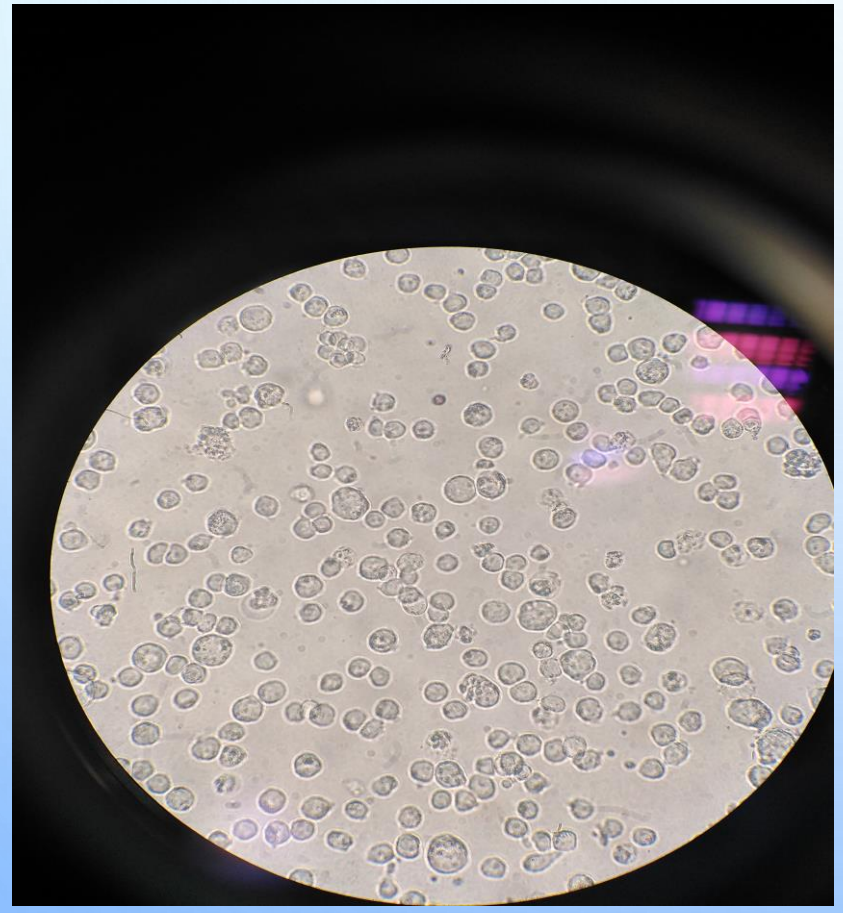
- Do not allow the cell concentration to exceed 1×10^6 cells/ml.
- **T-75** flasks are recommended for **subculturing** this product.
- **Medium renewal: every 2 to 3 days** (Do not change the culture medium of the cells earlier than every 2-3 days because they will be under severe stress)
- Before changing the culture medium and centrifuging, **pipet the cells well** to separate them from the bottom and walls of the flask and plate but do it **smoothly**.
- **Reagents for cryopreservation:** complete growth medium supplemented with **5%** (v/v) DMSO
- Storage at -70°C will result in loss of viability.

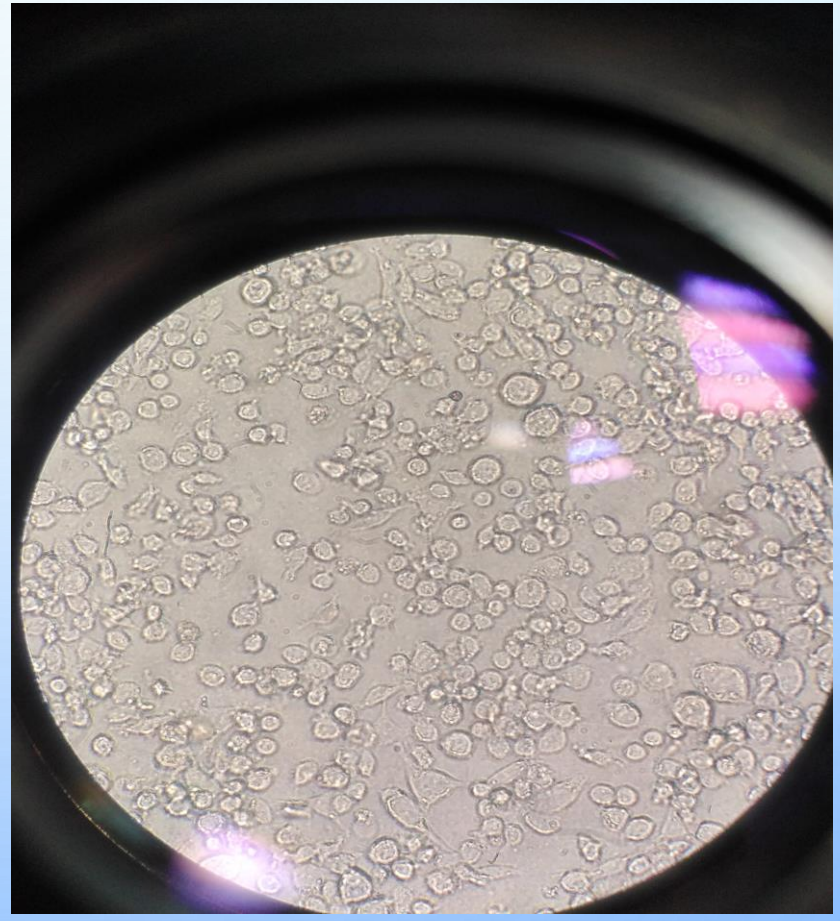
MICROSCOPIC PICTURE OF THP-1





MICROSCOPIC PICTURE OF THP-1






Thank
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