THP1 cells (ATCC TIB-202)

Version 2. Changes in red

Used for: phagocytosis assays

Origin: Human peripheral blood (1 year old boy with acute monocytic leukemia)

Monocytes; suspension cells

Storage: liquid nitrogen vapor phase

Base medium	**Final conc.**		
RPMI-1640 ATCC modified (Gibco **A1049101**) (stored in fridge)		500 ml	50 ml
Contains:			
L-glutamine (2 mM)			
Sodium Pyruvate (1 mM)			
Glucose (4.5 g/l)			
To make THP1 medium **add**:			
FBS (heat inactivated)	5%	25 ml	2.5
Pen & Strep 100× (aliquots in -20°C)	100 u/ml & 100 µg/ml	5 ml	0.5
β-mercaptoethanol, stock 55 mM (sterile, stored at 4°C)	0.05 mM	500 µl	50 µl
Total volume (ml)		555.5	55.5

Passaging:

Replace medium 3 days per week (afternoons)

Seed: 100,000 cells/ml on Friday and 200,000 cells/ml on Monday/Wednesday

Cells should not exceed 500,000 cells/ml

PASSAGING

1. **Look at the cells under the microscope**. Check for contamination (fungus, bacteria). Observe morphology and if cells are singular or in clumps. Always use gloves whenever you work with cells.

2. **Count the cells**. Pipette the cells up and down 5–10 × so you will count singular, separate cells only, not clumps. Using the same pipette, transfer a little bit of cell suspension into a new 0.2 ml tube. Use a p20 pipette to transfer 15 µl from this to be used for the cell count. Record number of viable cells/ml, viability % and size of viable cells.

3. **Passage cells**. Take a fraction from your cell suspension (briefly mix by pipetting prior to taking it out) and transfer to a 15 ml tube. This fraction should contain all the cells (total number, not concentration) that you want to seed into the new flask.

    a. **Mon/Wed**. Seed 200,000 cells/ml (cells will grow for 2 days (48h)).
    b. **Fri**. Seed 100,000 cells/ml (cells will grow for 3 days (72h)).

4. **Centrifuge**. Spin down the cells (use balance tube!) for 5 minutes at 130 × g (acc.9, dec. 5, RT), pipette off the supernatant (take 2 ml from this for mycoplasma testing if applicable) and resuspend the pellet in the desired amount of medium (volume that matches with the size of your culture flask).

5. **Grow** the cells at 37°C and 5% CO_2. Flasks should be in horizontal position.