Institut für HIV Forschung
SOP #26-00 **(April 2016 – BS)**

THP-1 Cell Lines

Reagents

Reagent	Vendor	Catalogue #
DMSO	Sigma	2650
RPMI-1640 media	Life Technologies	31870-074
L-Glutamine	Sigma	59202C-100ML
Pen/Strep (5000IU Pen/5000ug/ml strep)	Sigma	0781
FBS	Biochrom	S 0115
T75 Culture Flask	Oehmen	658 175
PMA (Phorbol 12-myristate 13-acetate)	Sigma	1585

Starting culture from frozen aliquot

1. Follow SOP "THAWING PBMCs", below is a summarized version (steps 2-6)
2. Warm R10
3. Thaw cells in water bath
4. Dilute in R10, spin 300xg, 5min
5. Resuspend in 5ml R10 in a 15ml conical
6. Count cells
7. Spin cells 300xg, 5min
8. Resuspend cells at $4x10^5$/mL in R20
9. Incubate at 37°C, 5% CO_2 for 4-7 days in the conical (vertical), count daily and check for exponential growth
10. Once growing exponentially, spin 300xg, 5 min
11. Resuspend in R10 at $3x10^5$/ml and transfer to
    - T75 flask (minimum 15ml, recommended 20-40ml) or
    - T25 flask (10-20mL)
12. Once the cells are in a constant state of exponential growth, culture them in 40mL of media in a T75 flask. If additional cells are required, larger flasks/culture volumes can be used

Culturing THP-1 cells (in a T75 flask)

1. Cells should have media exchanged every 3-4 days (every Mon/Fri) ideally.
2. Count cells. Cells are happiest between 2-$8x10^5$/mL
    a. When cell concentration is >$1x10^6$/ml, it is time to split them
3. We split our cells 1:10 by spinning the culture at 300xg, 5min and resuspending in 20mL R10.
    a. Note that THP-1 cells do not adhere strongly to the plastic so trypsin is not required. Pipette thoroughly to recover cells
4. Take 2mL from the resuspension and dilute in 38mL R10 in the culture flask. You can freeze the remaining cells or create additional cell lines
    a. THP-1 cells are good for about 25 passes (1-1.5 months) before they should be discarded. Also, if these cells show any sign of abnormal growth or you see excessive clumping when looking at them under a microscope then throw them away and start a new cell line

Freezing THP-1 cells
Follow SOP "FREEZING PBMCs"

Differentiating THP-1 cells
THP-1 cells can be differentiated into M1 type macrophages using PMA

1. Resuspend cells in culture flask and count
2. Remove number of cells needed (note that differentiated THP-1 cells do not divide)
3. Spin aliquot at 300xg, 5min
4. Resuspend at $4x10^5$ cells/mL in R10 and plate them:
    a. 24 well plate : 1mL/well
    b. 12 well plate : 3mL/well
    c. 6 well plate : 5mL/well
5. Add between 50-100ng PMA/mL culture and mix with P1000 pipette
6. Incubate at 37°C, 5% CO_2 for 24-72hr
7. Perform desired experiments on your new macrophages
    a. NOTE that macrophages are <u>extremely</u> adherent and require one of 3 things to get into suspension:
        i. Cell scraper
        ii. Trypsin
        iii. Or non-enzymatic cell disassociation solution