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Culturing THP-1 Cells

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ABSTRACT

THP-1 cells are a human monocyte suspension cell line from peripheral blood of a 1 year old infant who had acute mnocytic leukemia.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

https://www.atcc.org/Products/All/TIB-202.aspx

Preparing Media

1 The base medium for this cell line is RMPI-1640

2 Reguired supplements:

- [M]**1 % volume L-glutamine**
- [M]**10 % volume Fetal Bovine Serum**

Most catalog numbers of RMPI-1640 contain L-glutamine, however, some do not. Ensure that it is in the media before using it for culturing.

3 Optional Supplements:

- [M]**1 % volume PenStrep**
- [M]**0.05 Milimolar (mM) 2-mercaptoethanol**

PenStrep is not required for THP-1 culturing, however, if you are having issues with bacterial contamination, it can be used at 1X.

2-mercaptoethanol is stated as a required component for complete RPMI-1640 medium, however, in our laboratory it is not standard practice to add it.

Cell Storage

4 Always store cells in liquid nitrogen. This is for both the original tube of cells from ATCC and any passages afterwards.

Preparation of Materials & Reagents

5 Place the media bottle in the **37 °C water bath** at least **00:30:00 prior to using**

6 Thaw cells at **25 °C (room temperature)** for **00:10:00** or **37 °C in a water bath** for **00:02:00**

Working from fozen cells

7 Sanitize all items going into the Biological Safety Cabinet with 70% ethanol

8 As soon as the cells are thawed, transfer the cells to a **15 ml conical tube** and add **10 ml of complete media**

Cells are stored with 5% DMSO, which can lyse cells if they are left for too long.

9 Pellet cells for **00:03:00 at 500g**

10 Discard supernatant

11 Resuspend cells by pipetting up and down 5X in **5 ml complete media**

12 Transfer cells + media to a T-25 flask

Incubation

13 Incubate cells at **37 °C and 5% CO2 and 80% humidity**

Feeding and Splitting

14 THP-1 cells replicate after ~26 hours. In practice, it takes 2 days for a true doubling.

15 Once cells have doubled OR when media has begun to change colour, it is time to add media, split cells into new flasks, or to spin down to remove all media

step case

Adding media

16 If concerned about cell concentration, perform a cell count

17 Double the total media volume with new complete media

18 Carefully mix the new media in by rocking the flask back and forth

19 Place the flask back in the incubator

step case

Splitting cells into new flasks

16 If concerned about cell concentration, perform a cell count

17 Once the volume limit is reached for the T-25 flask it is time to move to a T-175 flask

18 Remove all cell + media from the T-25 flask and transfer them to the T-175 flask

This has to be done with a **10 ml pipette** because the larger volume pipettes do not fit in the T-25.

step case

Spinning cells down to remove all media

16 If cells are appearing unhappy, it may be beneficial to remove all of the current media and dead cells and replenish with new complete media

17 Transfer all cell + media to 50mL conical tubes

18 Pellet cells for **00:03:00 at 500g**