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 monocytic leukemia.

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PARENT PROTOCOLS
In steps of
Cell Surface Mild Acid Elution of MHC-bound Immunopeptides

Preparing Media

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

Required supplements:

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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.

Optional Supplements:

- **1 % volume** PenStrep
- **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

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

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

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

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

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

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**Incubation**

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

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**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 15 includes a Step case.
   
   **Adding media**
   
   **Splitting cells into new flasks**
   
   **Spinning cells down to remove all media**

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**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

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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.

19 Double the total media volume with new complete media

20 Place all flasks in the incubator

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**

19 Discard supernatant

20 Resuspend cells by pipetting up and down 5X in new complete media.

Unless cells were at a drastically low cell concentration, resuspend cells in the same volume of media they were in before pelleting.
21 Transfer cells to a new T-flask

22 Place flask into the incubator