

Nov 01, 2019

## Culturing THP-1 Cells

DOI

[dx.doi.org/10.17504/protocols.io.y6gfzbw](https://dx.doi.org/10.17504/protocols.io.y6gfzbw)

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**Protocol Citation:** Teesha C Luehr 2019. Culturing THP-1 Cells. **protocols.io**  
<https://dx.doi.org/10.17504/protocols.io.y6gfzbw>

**Manuscript citation:**  
<https://www.atcc.org/Products/All/TIB-202.aspx>



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**Protocol status:** Working

**We use this protocol and it's working**

**Created:** March 13, 2019

**Last Modified:** November 01, 2019

**Protocol Integer ID:** 21416

**Keywords:** human monocyte suspension cell line, human monocyte suspension cell line from peripheral blood, acute monocytic leukemia, cell

## 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.

## Troubleshooting



## Preparing Media

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

2 Required supplements:

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

### Note

Most catalog numbers of RPMI-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 millimolar (mM) 2-mercaptoethanol

### Note

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

### Note

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 frozen 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

### Note

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% CO<sub>2</sub> 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

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## STEP CASE

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### Adding media 4 steps

- 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