Nov 01, 2019

Culturing THP-1 Cells

DOI
dx.doi.org/10.17504/protocols.io.y6gfzbw

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DOI: dx.doi.org/10.17504/protocols.io.y6gfzbw

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

Protocol status: Working
We use this protocol and it's working

Created: March 13, 2019

Last Modified: November 01, 2019

Protocol Integer ID: 21416

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.

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

Note

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 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 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

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% 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 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