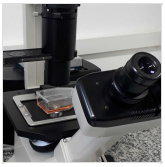

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THP-1 differentiation

Juliane C R Fernandes[1]

[1]Faculdade de Medicina da Universidade de São Paulo

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Juliane C Fernandes
Faculdade de Medicina da Universidade de São Paulo, Institut...

DISCLAIMER

ABSTRACT

Differentiation of THP-1 monocytic cell line in macrophage-like cells

PROTOCOL CITATION

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THP-1, monocyte, macrophage, differentiation, PMA, cell line

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PARENT PROTOCOLS

In steps of
Leishmania infectivity assessment using imaging flow cytometry

GUIDELINES

Differentiation of THP-1 monocytic cell line in macrophage-like cells.

MATERIALS TEXT

THP-1 monocytic cell line **ATCC Catalog #TIB202**

RPMI 1640 **LGC biosearch Catalog #BR30011-05**

FBS **Gibco, ThermoFisher Catalog #12657-029**

phorbol-12-myristate-13-acetate (PMA) **Sigma-aldrich Catalog #1002714643**

Trypan Blue Solution, 0.4% **Thermo Fisher Catalog #15250061**

Falcon Tube (50 mL) **Fischer Scientific** Step 1 PenStrep **Invitrogen - Thermo Fisher**

Neubauer Improved Haemocytometer Counting Chamber
Counting Chamber

Hawksley **AC1000**

Void depth: 0.1 mm
Counting Area: 1mm²

SAFETY WARNINGS

Recommended taking biosafety precautions.

DISCLAIMER:

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Plating and starting differentiation process 3d

1 10m

THP-1 monocytes **ATCC Catalog #TIB-202** cultivated in RPMI 10% FBS Pen/Strep in a humidified incubator at **34 °C** 5% CO_2. Carefully check for any contamination before initiating the protocol.

THP-1

Centrifuge monocytes **ATCC Catalog #TIB-202** at **200 x g, Room temperature , 00:08:00**

using Falcon Tube (50 mL) **Fischer Scientific**

Centrifuge
Benchtop Centrifuge
Eppendorf 5405000441
Any benchtop centrifuge will suffice

1.1 Estimate the number of bottles necessary to achieve enough cells for the experiment. After the first round of centrifuging, discard culture media and centrifuge the content of new bottles in the same tube. **go to step #1**

1.2 Resuspend pelleted cells in a volume sufficient for counting (usually 5 mL for each centrifuged bottle) 2m

1.3 Mix **10 µl** of the resuspended cells with **10 µl** of Trypan Blue. 2m

2 Count and evaluate cell viability placing **10 µl** of the 1:1 cell:Trypan mixture in a Neubauer Chamber 10m
Calculate the concentration of cell per mL (Medium of cells/chamber × 2 × 10^4)

2.1 Dilute cells/mL according to the preferred plate for the follow-up experiment

24 well plate - 1 × 10^6 cells/well - **1 mL** /well (flow cytometry)
6 well plate - 5 × 10^6 cells/well - **4 mL** /well (RNA extraction)

3 Add PMA.
Add PMA from the diluted stock (1 µg/mL). First, dilute 100x to obtain an aliquot of 10 ng/µL. Final concentration for THP-1 differentiation: 30 ng/mL diluted in RPMI 10% FBS.

3.1 Store for **72:00:00** at the incubator **34 °C 5% CO2** 3d

Resting 3d

4 Aspire cell culture media with a vacuum pump. Wash cells 3 times with PBS 1X to remove residual PMA.

5 Incubate cells with 1 or 4 mL - respectively for 24 well and 6 well plates - of fresh RPMI 10% FBS medium for further [3d]
72:00:00 **34 °C** **5% CO2**

THP-1 macrophages

6 Proceed to the assay of interest with fully differentiated THP-1 macrophages.

This protocol was tested to the following assays: miRNA inhibition, *Leishmania* infection, flow cytometry, and RNA extraction.
After differentiation cells stop duplicating and adhere to the bottom of plate wells (THP-1 monocytic cell line grow in suspension).