

SOP: SP068

Differentiating THP1 Monocytes into Macrophages

Materials and Reagents:

1. Culture of THP1 monocyte cells (see SOP: SP058)
2. 37 °C incubator with 5% CO₂
3. Water bath at 37 °C
4. RPMI 1640-Complete (see SOP: M023)
5. Phorbol Myristate Acetate (PMA) (Cat # P1585, stored at -20°C) (Note 1)
6. Table top centrifuge
7. Inverted Microscope
8. Tissue culture/biosafety cabinet
9. Serological pipettes
10. Falcon tubes 15 mL and 50 mL
11. T25, T75, flasks
12. Pipette-Aid

Protocol:

1. ____ Prepare the tissue culture Biosafety cabinet with materials (9-12) and turn on UV light at least 30 minutes. At the same time, warm RPMI1640-complete medium in a water bath 37°C
2. ____ Prepare cRPMI+PMA, adding PMA (final concentration 200 nM) to the RPMI1640-complete medium (Note 2).
3. ____ From a continuous culture of THP1 cells, transfer the cell suspension to a 15 mL flask and centrifuge at 125 x g, 5 min. (Note 3)
4. ____ Retrieve the tube from centrifuge, decontaminate it spraying with 70% ethanol, inside the cabinet decant and discard the supernatant.
5. ____ Re-suspend the cells in 2 mL of RPMI1640-complete medium. Count the cells using the hemocytometer (Note 4).
6. ____ Prepare a cell suspension at final concentration of 3×10^5 live cells/ml using the previously prepared RPMI+PMA and incubate at 37 °C with 5% of CO₂ for 72 hours. (Note 5).
7. ____ After 24 hours check the cells under inverted microscope, assessing the adherence. Place the flask in the incubator up to complete 72 hours.

Notes:

1. The stock concentration of PMA is 1 µg/µL equal to 1.62×10^6 nM
2. The Molar concentration of Stock PMA is 1.23×10^{-4} . To prepare 10 mL final vol, multiply 10,000 uL per 1.23×10^{-4} and solve for the volume of PMA from the stock (in this case 1.2 uL). For T25 culture flask mix 10 mL of RPMI1640-complete medium and 1.2 µL from PMA stock. For T75 culture flask mix 20 mL of RPMI1640-complete medium and 2.4 µL from PMA stock.
3. To set 125 x g set 750 RPM approximately, in the *Allegra Centrifuge* located at C 210 Lab.
4. To count the cells use SOP: SP067.
5. At this point the cell have starting become macrophages and they will be adhered and covering more than 95% of the bottom of the flask.