SOP: SP058

Culturing of THP1 Monocytes

Materials and Reagents:

1. THP1 cells, monocyte (ATCC Cat# TIB-202) (stored in liquid Nitrogen)
2. 37 °C incubator with 5% CO_2
3. Water bath at 37°C
4. RPMI 1640-complete (See SOP: M023)
5. Trypan blue solution (0.4%, SIGMA Cat #: T8154)
6. Hemocytometer
7. Inverted Microscope
8. Light microscope (objective lens 10X)
9. Table top centrifuge
10. Filtration Unit 0.22 μm and vacuum pump.
11. Sterile 10 μL tips
12. Pipette 10 μL and pipette-Aid
13. Falcon tubes 15 mL and 50 mL
14. T25, T75, T125 flask
15. Serological pipettes 5, 10, 25 mL
16. Electronic pipet-Aid

Protocol:

1. ____Prepare the tissue culture Biosafety cabinet with all materials (points from 10 to 16) and turn on UV light at least 30 minutes. At the same time, warm RPMI1640-complete medium in a water bath 37 °C
2. ____Remove the THP1 cells from liquid nitrogen and thaw in the water bath 37 °C (approximately between 2 to 4 minutes) (Note 1).
3. ____As soon as the cells are thawed, remove from the water bath and decontaminate it spraying with 70% ethanol. At the same time, remove the RPMI1640-complete medium from the water bath, decontaminate spraying with 70% ethanol and put the cells and medium into the cabinet.
4. ____Transfer the THP1 cells to 15 mL Falcon tube containing 9.0 mL of RPMI 1640-complete medium and mix slowly inverting the tube.
5. ____Centrifuge the cells at 125 x g for 5 minutes (Note 2).
6. ____While cells are spinning, prepare a T25 flask with 5.0 mL RPMI1640-complete medium.
7. ____Retrieve the tube from centrifuge, decontaminate it spraying with 70% ethanol, inside the cabinet decant and discard the supernatant.
8. ____Resuspend the cells in 1 mL of RPMI1640-complete medium and transfer to the prepared T25 flask (Final volume 5 mL).

9. ____Incubate the T25 flask at 37 °C with 5% CO_2 (Note 3). The cells recently thaw must be place in 5 mL and must be incubated placing the T25 flask vertically. Check cells daily:
    a. Macroscopically

i. Color media from orange to yellow, the media is beginning to turn acidic. The cells need to be fed.
ii. Turbidity: very high turbidity or cloudiness will suggest bacterial contamination. (discard the culture)

10. ____Every 2-3 days remove all suspension cells from T25, transfer to 15 mL Falcon tube and centrifuge at 125 X g for 5 minutes (Note 5).
11. ____Retrieve the tube from centrifuge, decontaminate spraying with 70% ethanol, decant and discard the supernatant. Resuspend the cells in 2 mL of RPMI1640-complete medium. Count the cells using the hemocytometer using SOP:SP067
12. ____Maintain the culture mixing the cells suspension with fresh RPMI1640-complete medium. **Recommended inoculum:** 2 to 4 x 10^5 viable cells/ml. (Note 6)
13. ____Return culture flask to 37 °C incubator with 5% CO_2.
14. ____Subculture or upscale when cell concentration reaches 8 x 10^5 viable cells/mL. For upscaling to a T75 culture flask add the 2 mL cells suspension obtained from T25 flask to 23 mL of RPMI1640-complete medium. For upscaling to a T125 culture flasks add the 2 mL cells suspension from T75 flask to 48 mL of RPMI-1640-complete medium.

Notes:

1. To reduce the possibility of contamination, keep the O-ring and cap out of the water.
2. To set 125 x g set 750 RPM approximately, in the *Allegra Centrifuge* located in C 210 Lab.
3. If using vented cap flask: screw the lid on firmly; if not, make sure the cap is loose to allow CO_2 exchange in the incubator.
4. The THP1 cells monocytes grow suspended in the medium, they do not attach to bottom of the flask. If during those days the medium is turning acidic (if the phenol red indicator in the medium turns orange or yellow), change the medium following the steps 4 to 9. The first time (after thawing) the cells must be incubated for 72 hours prior to changing the media.
5. Transfer 10 µl of cells suspension to a 0.65 tube. To avoid contamination do not introduce the pipette body inside the tube, instead of this, place the 15 ml Falcon almost in horizontal position and take the cells just introducing the sterile tip Follow the SOP to Count cells with Hemocytometer.
6. Do not allow the cell concentration to exceed 1 x 10^6 cells/mL.