SOP: SP066

Culturing of J774a.1 macrophages

Materials and Reagents:

1. J774a.1 cells, monocyte/macrophage macrophage (ATCC Cat# TIB-67)
2. 37 °C incubator with 5% CO_2
3. Water Bath 37 °C
4. cDMEM media (See SOP: M022)
5. Tissue culture/biosafety cabinet
6. Table top centrifuge
7. Hemocytometer
8. Inverted microscope
9. Roller bottle apparatus
10. Trypan blue (0.4%)
11. PBS 1X
12. Serological pipettes
13. Cell Scraper (BD #353085)
14. 10 μL tips/pipetman
15. 0.65 snap cap tubes
16. Falcon tubes – 15 mL
17. Falcon tubes, 50 mL
18. T25, T75, T125 flask (TC treated)
19. Roller bottles

Protocol:

1. _____ Prepare the Biosafety cabinet with all the materials from 11 to 18. (Using aseptic technique. Let on the UV light at least 30 min before starting the process).
2. _____ Remove J774a.1 cells from Liquid Nitrogen.
3. _____Immediately transfer cell line to 37 °C water-bath. It takes about 2-4 minutes to defrost. Warm cDMEM media as well.
4. _____When the cells are thawed, aseptically transfer the cells to 10 mL of cDMEM in a 15 mL conical.
5. _____ Centrifuge cells at 500 x g at room temperature for 10 min. (1200 RPM in the Allegra 6R centrifuge in C210)
6. _____While cells are spinning, prepare a T25 flask with 9 mL of cDMEM.
7. _____ Retrieve cells from centrifuge, decant supernatant, and discard. Clean the tube before put it again inside the cabinet with ethanol 70%.
8. _____ Re-suspend cells in 1 mL of fresh cDMEM and transfer to the prepared T25 flask
9. _____ Incubate the cells in the 37 °C incubator with 5% CO_2 (Note 1)
10. _____ Check cells daily to see if they need to be fed:
    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)

   b. Microscopically: Using the 10X objective in the inverted microscope evaluate the cellular confluence. When cells reach 75% of confluence, the cells need to be fed and split.

11. _____To feed the cells, place the T25 flask vertically and using a 10 mL pipette remove the media, wash cells adding 10 mL of 1X PBS, scrape and transfer the cell to a 15 mL Falcon tube, centrifuge as step 5 then perform step 6 and re-suspend it in 2 mL of fresh media. Count cells under a hemocytometer (notes 2 & 3).
12. _____ Cells that are being fed are usually split 1:10 or 1:20 (Note 4).
13. _____ Make note on flask that it has been fed and return cells to 37 °C incubator with 5% CO_2.
14. _____When media begins to turn acidic, upscale from a T25 to two T75: to do this, follow the step 11 and start the new T75 flask containing a final volume of 25 mL RPMI complete media plus cells (Note 5).
15. _____ Feed the original T25 and make note on flask that it has been fed. Return cells to 37 °C incubator with 5% CO_2 (Note 6)
16. _____When the media begins to turn acidic again, upscale to two T125 each containing a total volume of 50 mL of media plus all cells from the T75 flask.
17. _____ Return cells to 37 °C incubator with 5% CO_2.

Notes:

1. If using vented cap flasks: screw the lid on firmly, if not using vented cap, make sure the cap is loose to allow CO_2 exchange in the incubator.
2. Take two aliquots of 10µl of cell suspension (each aliquot in a 0.65 snap cap tube). You must be VERY careful when removing the cells to avoid contamination of the cells. Place the falcon tube horizontally until you reach the cell suspension only allowing the sterile tip to touch the cells. There is an SOP SP067 explaining in detail how to count the cells.
3. The cells will grow best when they are between 4×10^5 and 7×10^5. Cell greater than this density should be expanded to new flasks.
4. This means that the cell density must be adjusted to be between 5×10^3 and 2×10^4 cells per mL. The fewer the cells the longer they will take to recover, but usually the density will be back up in 2-4 days.
5. Always keep a T25 flask culture as a backup in case of contamination.
6. At this point: use T75 for infection experiments; continue to upscale if cells are being used for exosome production.