# CULTURING OF RAW 264.7 MACROPHAGE

## AIM

To culture Cell line called RAW 264.7 Macrophages to conduct various in-vitro experiments on mammalian cells.

## REQUIREMENTS

1. DMEM Media with 10% FBS
2. Phosphate Buffer Saline (PBS)
3. Culture Dishes (60 mm plate/35 mm Dish/T25 flask/T75 Flask)
4. Mini-cooler
5. Falcon tubes (50 mL and 15 mL)
6. Centrifuge
7. Pipettes
8. CO2 incubator
9. BSL safety level-2 culture hood with HEPA
10. Water Bath
11. Cell scraper
12. Pipette Gun

## PROCEDURE

### A) RAW 264.7 Macrophage Reviewing From Glycerol Stock:

- Switch on the BSL Safety level 2 cell culture hood, and turn on the UV for 30 minutes.

- Take out the vial containing the Glycerol stock from the liquid nitrogen and place it in the mini cooler( -20 °C).
- Take the stock inside the hood and dilute the stock with warm DMEM media.
- Now pellet down the cells using the centrifuge at 1300 g for 5-10 minutes, discard the supernatant and resuspend the cells in 5 mL warm DMEM media.
- Take 100 uL of the cells and add 100 uL of formal saline in 1:1 ratio, take 200 uL of this mixture onto the hemocytometer and count the no. of cells.
- Depending upon the no. of cells wanted for the experiment, add the stock to the dish and fill with media to fill the capacity of the dish.
- Properly spread the cells and now place the culture dish in the incubator.
- For RAW 264.7 Macrophages, it will take 3-4 days to reach 90% confluency.

B) Media Change and cell splitting:

- DMEM media consists of phenol red which acts as a pH indicator. if the media turns into light red or yellow color, the media should be immediately changed.
- Take the dish out of the incubator, remove the old media with a pipette and add 1-2 mL of PBS/fresh media slowly by adding along the walls. Shake it slowly and remove the PBS/media with the help of a pipette.
  This step is done to remove the dead cells and unattached cells.

- Now add the new media depending upon the size of the dish.
- During cell splitting, after washing the cells with PBS/fresh media, add 2 mL of fresh media and dislodge the cells from the surface of the culture dish using a cell scraper.
- Take 100 uL of the dislodged cells and 100 uL of formal saline in a 1:1 ratio and place the mixture on the hemocytometer to count the cells.
- Calculate the no. of cells per mL and seed the cells into the new culture dish (30%-40%) by adding the required volume of the dislocated cells and add the fresh media. Spread the cells properly.
- Place the culture dishes in an incubator at 37 °C with 5% CO2.
- After 2-3 days we can get 90% confluency with which the proceeding experiments can be conducted.

C) Cell Freezing and Storage:

- Take the culture dish with 90-95% cell confluency,
- Remove the old media and give a PBS wash. Then add 2 mL of media and dislodge the cells with the help of a cell scraper.
- Take the dislodged cells in a 15 mL falcon tube, add 8 mL of media, then pellet down the cells.
- Discard the supernatant, resuspend the cells in a solution mixture containing 70% DMEM media, 20% FBS and 10% DMSO and transfer the cells along with this mixture into the cryovial.

- Place the vials in Mr. Frosty, in which temperature drops gradually causing less stress to cells. After 24 hours, place the cryotubes in the liquid nitrogen tank.