TEM for cultured cells.v2

Procedures:

1. Prepare cells in T75 flask or equivalent surface area for TEM (Myoblasts should be prepared in 2 T75 flasks whilst myotubes are prepared in 1 T75 flask).

2. As cells are ready for fixation, remove medium from flask, wash cells with 10 ml 1X PBS at room temperature for twice, 5 minutes each.

3. Add 10 ml of room temperature 2.5% glutraldehyde/1X PBS at pH 7.2 into the cultured cells, transfer the flasks to 4ºC for 2 hours. (**IMMPORTANT NOTE:** To make 2.5% glutaraldehyde, dilute 10X PBS to 2X (or make 2X PBS) and adjust pH to 7.2. Dilute glutaraldehyde to 5% in water. Mix 2X PBS and 5% glutaraldehyde 1:1 to make a final 2.5% glutaraldehyde in 1X PBS at pH7.2)

4. Thoroughly remove fixative from the cultured cells, wash cells with 10 ml 1X PBS for 3 times, 5 minutes each at room temperature.

5. Add 3 ml 1X PBS to the fixed cells, carefully scrape the cells off with a cell scraper, transfer the cell suspension to a 15 ml centrifuge tube.

6. Add another 3 ml 1X PBS into cultured cells, repeat procedure as described in Step 5.

7. Centrifuge cell suspension at 2000 rpm for 3 min, carefully remove PBS (by discarding or pipetting) without affecting the cells.

8. Resuspend cells in appropriate amount of 1X PBS (1.5ml for myotubes and 500 µl for myoblasts) and store cells in 4ºC (maximum storage of 1 week) before proceeding to the next steps.

9. Wash cells with 1X PBS for twice, 5 minutes each at room temperature. (Spin cells at 2000 rpm for 3 min).

10. Post-fix in 1 % *OsO4,* pH 7.4 for 1 hour at room temperature in a fume hood.

11. Wash twice in 1X PBS for 5 minutes (spin the sample at 2000 rpm for 5min) at room temperature.

12. Prepare gelatin solution (6-10%) keep gelatin at 60ºC (gelatin must be well dissolved).

13. Spin the sample down at 2000 rpm for 3 min. Remove the PBS. Add in the gelatin to cover the pellet. Mix well and repeat spin as above.

14. Remove the excessive gelatin, place the sample at 4ºC for 10-15 min to solidify the gelatin, test it by a tooth pick.

15. Add fixative (2.5% glutraldehyde) to fix the gelatin for 10 min, wash twice, 5min each with distilled water. Trim the sample to about 1-2 mm cubes.

16. Store the cells sample in 1 ml 1X PBS at 4ºC before proceeding to next step.