Subcellular Fractionation

Prepare buffer A:

10 ml buffer A

50 ul PMSF (1/200)

50 ul Benzamidine (1/200)

20 ul Leupeptine (1/500)

50 ul Aprotinin (1/200)

Wash and scrape cells. Procedures may vary in this step based on the cell type and what is needed for the final samples.

Spin cells at 4ºC for 2 min at 2000 rpm

Wash quickly in buffer A solution

Spin again at 4ºC for 2 min at 2000 rpm

Resuspend cells in 200 ul buffer A solution

Leave on ice for ~20 min

Aspirate up and down with a 1 ml syringe/25 gauge needle 15 times

Check sample on microscope with trypan blue to verify that fractionation has taken place

Spin at 4ºC for 3 min at 3000 rpm

Pipette out supernatant (cytoplasm) and transfer to new eppendorf tube

Resuspend pellet (nuclei) in buffer A solution and spin again at 4ºC for 3 min at 3000 rpm repeating this step for a total of 3 times, discarding the supernatant each time

Resuspend pellet in 50 ul buffer A

Sonicate all samples and take the OD as stated in "Western Blot Protein Preparation" protocol