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Mammalian Cell Fractionation Protocol

Reagents:
CSK Buffer:
20 mM HEPES-KOH, pH 7.6
40 mM Potassium Glutamate
1mM $MgCl_2$
1mM EGTA
300 mM Sucrose
1mM DTT
Protease Inhibitors

Protocol:

(1) Plate cells at desired concentration prior to experiment on 10cm Tissue Culture Dishes

(2) Prepare CSK buffer, utilizing a total of 600uL per each 10cm Tissue Culture Dish (make CSK buffer fresh for each experiment)

(3) To (# plates x 250uL) CSK Buffer, add 0.1% Triton-X-100, mix at 4°C until Triton is mixed evenly throughout buffer.

(4) Harvest Cells, first rinse in ice cold 1x PBS, scrape cells into 1mL 1xPBS, pellet by centrifugation. Rinse pellet 1x with 1x PBS, and fully aspirate all remaining PBS.

(5) Add 250uL of CSK Buffer + Triton-X-100 to each pellet, mix thoroughly via pipetting up and down, then brief vortexing, taking care not to heat solution.

(6) Return to ice for 20 minutes, occasionally and briefly mixing solution.

(7) Centrifuge CSK/Cell Mixture at 4°C, 1,500RCF, for 3 minutes.

(8) Carefully remove Supernatant via pipetting – this is your Soluble Triton Fraction – transfer to 1.5mL Eppendorf Tube

(9) Add 200 uL CSK Buffer (<u>without</u> Triton-X-100) and thoroughly resuspend pellet via vortexing and pipetting.

(10) Run Protein Concentration Assay to determine the concentration of the Soluble Fractions, only (the insoluble fractions contain flocculant, making Protein Concentration Assays unreliable).

(11) To each Soluble Fraction, add 90uL of 3x SDS-PAGE Loading Buffer (with BPB and β-ME). Add enough 1x SDS-PAGE Loading Buffer to equalize Soluble Fraction protein conc.

(12) In Insoluble Fraction, perform the same loading buffer addition and equalization (keep in mind that volume is less) in proportion to soluble fraction. Insoluble fraction must be mixed with force to break up as much flocculant as possible.

(13) Boil all fractions at 100° C for 5 min.

(14) Load into SDS-PA gel and perform same WB protocol as cell lysates.