

Cellular Protein fractionation for Mammalian Cells

Cell harvest and lysis

- 1) Collect cells and incubate in hypotonic lysis buffer (20mM Tris pH 7.5, 5mM MgCl₂, 5mM CaCl₂, 1mM DTT, 1mM EDTA, protease inhibitor). Incubate in lysis buffer for 30 minutes on ice.
- 2) Dounce homogenize the cells (40 strokes) and view cells under the microscope to verify that the membranes have been removed from the cells.
- 3) Spin cells (4°C) for 20 minutes @ 3000 rpm.

Isolation of microsomal proteins

- 4) Remove the supernatant, which contains the cytosolic and microsomal proteins. The microsomal fraction is isolated from the cytosolic fraction by ultracentrifugation (100,000 x g) for ~3 hours. The resulting microsomal pellet is solubilized in a denaturing buffer (50mM Tris pH 8.3, 5mM EDTA, 0.05% SDS, and 6M urea).

Isolation of cytosolic proteins

- 5) The cytosolic fraction is TCA precipitated from the supernatant, followed by resuspension in the denaturing buffer.

Isolation of nuclear proteins

- 6) The nuclear pellet from step 3 is resuspended in 1 ml low-salt buffer (20mM Tris pH7.5, 5mM MgCl₂, 20mM KCl, 1mM DTT, 1mM EDTA). Add 100 µl high-salt buffer (20mM Tris pH7.5, 5mM MgCl₂, 1.2M KCl, 1mM DTT, 1mM EDTA) and mix. Add high-salt buffer repeatedly for a total of 10 times. The mixture is incubated with gentle agitation for 2 hours at 4°C. Centrifuge at 25,000*g for 15 min. Discard the pellet and precipitate the supernatant by TCA. Resuspend the TCA precipitated pellet in the denaturing buffer.

This method is used for selectively removing nuclear proteins without removing the histones from the DNA. The method also leaves the genomic DNA behind, which simplifies subsequent analyses.

Protein assay

- 7) Perform protein assay on cytosolic, microsomal and nuclear fractions using BCA protein assay kit (Pierce).