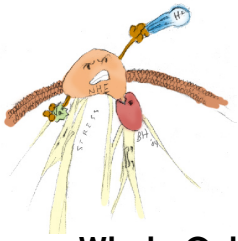
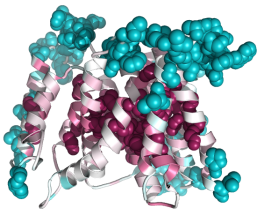


PROVOST & WALLERT RESEARCH

Investigating the Biochemistry and Cellular Physiology of NHE1

EST. 1998



Whole Cell Lysate Preparation using RIPA Buffer – for membrane and soluble proteins

RIPA Buffer (Radio-Immune Precipitation Assay) is used to lyse cultured cells to prepare protein extraction from cytoplasmic, membrane and nuclear proteins. Samples prepared with RIPA Buffer can easily be used with a BCA protein assay, western blot, immuno assays or other biochemical determintion. To maintain proteins in the state at time of lysis it is critical to keep cells on ice and only use ice cold buffer throughout to reduce protease, kinase, phosphatase or other enzymatic activity of lysates.

T-25 flasks:

1) Remove old media and rinse cells with 2-3 ml of *ice cold* PBS.
2) Tilt flask 1-2 min on ice to drain residual PBS and remove by aspiration.
3) Add 0.5 ml of ice cold PBS and scrape cells. Transfer to labeled chilled microfuge tube.
4) Rinse cells with additional 1 ml of PBS and add to scraped cells.
5) Centrifuge at 2,500 x g for 5 min at 4oC. Decant supernate, keep pelleted cells.
6) Resuspend pellet in 0.25 ml of RIPA Buffer – use a pipet tip to suspend cells.
7) Lyse cells by sonication for 2, 30 second pulses (50% power) while on ice.
8) Shake mixture gently on ice for 15 min.
9) Centrifuge samples at ~14,000 x g for 15 min to pellet cell debris. Keep supernatant soln.
10) Perform BCA (Pierce) NOT Bradford/biorad protein assay.

RIPA Buffer

50 mM Tris-HCl, pH 7.4,
150 mM sodium chloride,
1 mM ethylenediaminetetraacetic acid (EDTA),
1% NP-40
1% Sodium Deoxycholic acid,
0.1% sodium dodecylsulfate (SDS),

1 mM phenylmethylsulfonyl fluoride, (add fresh)
Protease Inhibitors (add fresh - see manufacturer's instructions)