PROVOST & WALLERT RESEARCH

Investigating the Biochemistry &
Cellular Physiology of NHE1
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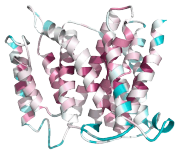
E. Coli Lysis

Purification Protocol



Cell lysate preparation.

Resuspend Pellet Volumes: 20 - 100 ml culture - resuspend in 2-10 ml; 100-500 ml 10-25 ml; 1-2 liter 25-50 ml

- Resuspend in the appropriate binding buffer (GST or His purification) with the following additions
    - for >500 ml cultures. Add 100 ul of 5 mg/ml DNAseA (DnaseA 0.003 mg per 1 liter pellet or 2 ul of 25000U/ml per ml). If using Pierce DNase I cat 89836, use 5 ul for a liter pellet. May need to add 5mM MgCl2 and 130 uM Ca+2 for optimal activity.
        - Optional 100X DNase I reaction buffer 100 mM Tris pH 75, 250 mM MgCl2, 10 mM CaCl2, 500 mM EDTA.
    - Add EDTA free protease inhibitor as per manufacture's instruction. Once dissolved, the half life is quick so do not add until ready. OR 1 mM PMSF depending on material access
    - 10 mM β (2-) mercaptoethanol *** Do not add until the day of use. (*pure β ME is 14 M*)
    - Depending on the pellet viscosity you may need to sonnicate to get the pellet fully suspended.

AutoLysis Instructions: Resuspend pellet up and down with a transfer pipette until the mixture is homogeneous

- Freeze on ice or isopropanol/dry ice bath. Thaw, resuspend and freeze a second time.
- Fully resuspend pellet with disposable pipetor.
- Centrifuge at 8,000 x g for 20 min at 4oC.
- Transfer supernatant to clean 50 ml falcon tube(s). Continue purification.

Standard, Non - Autolysis Instructions

- Add Lysozyme (final conc of 1.0-0.5 mg/ml] - Refreeze unused lysozyme).
- Incubate on ice for 30 min while rocking of resupending manually.
- Sonnicate ON ICE for 3 x 1-3 min bursts as high as your sample can take without cavitation.
- Centrifuge at 8,000 x g for 20 min at 4^{o}C. NOT in falcon tubes. Use the smaller polypropylene tubes. Must be filled at least halfway to centrifuge at this speed.
- Transfer supernatant to clean 50 ml falcon tube(s). Continue purification.