MDH Lysate Preparation Protocol:

Bacterial Lysis for Recombinant Protein Purification

***Lysate Buffer Resuspension Volume*:** 20 - 100 ml culture - resuspend in 2-10 ml; 100-500 ml 10-25 ml; 1-2 liter 25-50 ml

Lysis Buffer: Resuspend in the same column binding buffer used for His-tag protein purification with the following additions

Basic His-Tag Protein Lysis Buffer

- 50 mM Tris-Cl (pH 8.0)
- 1 mM Imidazole (not critical but helps reduce non-specific protein binding to Ni column)
- 100 mM NaCl
- 0.1 mM EDTA

Add the following to the Lysis buffer as indicated:

- DNAse A – should be used only 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 βME is 14 M*)
- Optional - 0.1% Triton X-100 (Optional - *for prevention of aggregation of hydrophobic and membrane proteins). Detergents chosen for the lysis solution should be specific to the proteins*. Not needed for most MDH proteins.
- Depending on the pellet viscosity you may need to sonnicate to get the pellet fully suspended.

Standard Bacterial Cell Lysis

- Add Lysozyme (final conc of 1.0-0.5 mg/ml] - Refreeze unused lysozyme).
- Incubate resuspended on ice for 30 min while rocking / resuspending 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°C. NOT in falcon tubes. Use small ~30 ml polypropylene tubes. Tubes must be filled at least halfway to centrifuge at this speed.
- Transfer supernatant to clean 50 ml falcon tube(s). Continue purification.

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

XJ Autolysis™ *E. coli* strains are an alternative for bacterial transformation and lysis from Zymoresearch. These strains are efficiently lysed following arabinose-induced expression of the bacteriophage λ endolysin protein, coupled to a single freeze-thaw cycle. XLa Autolysis cells are JM109 cells with the gamma lysozyme gene under arabinose promotor. XJb Autolysis cells are BL21 cells with gamma lysozyme gene under the arabinose promotor. Both XJa and XJb come with or without DE3. Induce with 3 mM L-arabinose with 1 mM $MgCl_2$. (make as a 500 or 1000X stock)

For optimal lysis, more care needs to be taken when selecting the lysis buffer. However, even very low concentrations of a detergent improve lysis significantly XJb is ideal for recombinant protein expression.

For this method to work, arabinose must be included when inducing protein with IPTG

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