

Buffer components and homogenization protocol

Homogenization buffer (final conc)	BASE BUFFER - Make 100 ml of base
50 mM Tris-Cl pH 7.1	-all but PMSF and DTT – must add these fresh each time.
20 mM Sodium Fluoride (NaF)	- from higher conc stock
50 mM β glycerophosphate	- from solid – on research shelf
0.5 mM Na Orthovanadate	- from solid on research shelf
300 mM Sucrose	- from frozen concentrated stock – 200 mM
1 mM EDTA	- from solid research shelf
10 mM sodium metabisulfite (not –ate)	- from liquid stock in biochem lab or research space
1 mM PMSF (or protease cocktail pellet)*	- from solid research shelf
1 mM DTT**	* if using PMSF I would make fresh, old stuff in lab is OLD Otherwise use tablet when using. Use manufacture's instruction – look up on 'et ** Make a 1 M stock (1 ml, and freeze in 100 μ l aliquots) look in freezer for solid to make stock. This is smelly...

5 - 50 μ g protein loaded in the lane.

Note – most papers do not tell you how much buffer to add per heart. I would start with a “junk” heart, check mass and practice. See what the protein concentration is after following the protocol below. You may need to adjust the volume of buffer per mass of tissue up or down depending on the final concentration of protein. i.e. if the protein concentration of your homogenate is less than 1.0 mg/ml. use less homogenation buffer or more tissue. If the protein concentration is greater than 5.0 mg / ml you can add a bit more, but only if the volumes are too small to deal with... (Handling thing not a chemistry thing).

Homogenization Protocol:

1. Freeze tissue in LN2 and record mass.
2. Incubate pestle in -70° prior to pulverizing tissue. Add a bit of LN2 to pestle while grinding to keep things cold.
3. Transfer powdered material to an ice cold dounce homogenizer and add 0.015 ml of homogenization buffer per mg of tissue directly to homogenizer.
4. On ice, (to avoid heat buildup) homogenize the tissue with 3-5 passes with the drill press.
5. Separate 50 μ l of sample from aliquot for protein assay. This allows you to do a protein assay without freeze thawing your sample. Freeze the main portion of your lysate
6. Calculate the protein concentration and bring protein to 1.0 mg/ml total protein. (take a bit of the homogenate – not necessarily all of the soln – and add enough homogenization buffer to bring to 2.0 mg/ml.
7. Add and equal amount of 2X SDS PAGE Sample buffer = protein concentration now is 1.0 mg/ml (or 1.0 μ g/ μ l). Heat to 90 $^{\circ}$ C for 5-10 min. Centrifuge at max rpm for 5 min to remove particulate. Load protein on gel. 5 μ g – 50 μ g depending on protein and antibody.