**Myofibril Prep**

**from Human Ventricular Tissue / Mouse Heart Tissue**

**Rigor Buffer (20mL)**

10 mM Tris pH 7.0 (1.3M stock): 154 uL

5 mM EGTA (0.5M stock): 200 uL

132 mM NaCl (2M stock): 1.32 mL

5 mM KCl (2M stock): 50 uL

1 mM MgCl2 (1M stock): 20 uL

1 mM NaN3 (2M stock): 10 uL

*1 mM DTT (1M stock): 20 uL*

*1 ug/mL leupeptin (10mg/ml stock): 2 uL*

*1 uM Pepstatin A (5mg/ml stock): 3 uL*

*0.1 mM PMSF (0.2M stock): 10uL*

**Wash Buffer (20mL)**

30 mM Imidazole pH 7.0 (1M stock): 600 uL

60 mM KCl (2M stock): 600 uL

2 mM MgCl2 (1M stock): 40 uL

1 mM NaN3 (2M stock): 10 uL

*1 mM DTT (1M stock): 20 uL*

*1 ug/mL leupeptin (10mg/ml stock): 2 uL*

*1 uM Pepstatin A (5mg/ml stock): 3 uL*

*0.1 mM PMSF (0.2M stock): 10uL*

\*Note: before adding inhibitors (*italicized*), bring total volume to 20 uL with dH20 and pH to 7.0

**Wash Buffer + Triton (5mL)**

Wash buffer: 4.875 mL

0.5% Triton-X 100 (20% stock): 125 uL

**Procedure:**

1. Grab two buckets of ice (one for samples, one for homogenization)
2. Set up homogenizer to cool, and cool down centrifuge
3. Prep and pH Buffers (Rigor, Wash, Wash + Triton, .2M ATPase)
4. Thaw and Weigh Heart Tissue Weight = \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
   1. Cut heart into small pieces
5. Homogenize heart tissue in 4 mL of Rigor Buffer for 10 sec at 9,000rpm with electric homogenizer
   1. Cool homogenate on ice for 30 seconds
   2. Repeat homogenization and cooling on ice three more times, for total of 4x
6. Centrifuge homogenate 2,500 xg for 5 minutes.
   1. During this time, clean homogenizer (water x3, pick with tip, end w/ 70% EtOH)
7. Resuspend pellet in Wash Buffer plus Triton X, incubate for 5 minutes, resuspending every minute. Centrifuge 2,500 xg for 5 minutes.
   1. (Note: Consider starting MG/AM prep during one of these CF spins)
8. Resuspend the pellet with Wash Buffer (no Triton X) and centrifuge at 2,500 xg for 5 minutes. **Repeat three times, re-using same pipette tip to conserve protein.**
   1. Turn on spectrophotometer (15-20 min to warm up) and prep cuvette
9. Resuspend pellet in the working buffer (ATPase for ATPase Buffer) and measure the concentration.
   1. **Important:** Re-suspend in 500-750 uL buffer/tube, and then marry the two tubes for total volume of 1-1.5mL (pending size of pellets)
10. Measure concentration of myofibril: dissolve myofibril in warm 1% SDS in water bath, measure absorbance at 280 nm, 320 nm, and 700 nm.
    1. Extinction coefficient: 0.7 ml/mg-cm. (Knight, P.J. & Trinick, J.A., 1982)

|  |  |
| --- | --- |
| Blank | Sample |
| 450 uL dH20 | 450 uL dH20 |
| 25 uL ATPase buffer | 25 uL myofibril |
| 25 uL 20% SDS | 25 uL 20% SDS |

A280 =

A320 =

A700 =

[myofibril] = (A280 - A320)/0.7 x 20 = \_\_\_\_\_\_\_\_\_\_\_\_\_ mg/mL (10 mg/mL is ideal for ATPase)

1. **Set aside 100 uL of myofibril (in ATPase buffer) for mass spec analysis**
   1. **Place into box -20°C**
2. Option to continue with myofibril ATPase (MG/AM) or freeze with Glycerol 1:1
   1. 1:1 Freeze: Combine equal parts of Rigor Buffer and Glycerol
   2. Keep on ice for ~5 minutes
   3. Spin down desired volume of myofibrils to pellet (to be kept for imaging)
   4. Resuspend these myofibrils in cold 1:1 rigor/glycerol
   5. Note: Try to save at least 500 uL for myosin extraction