Acetone Powder from Rabbit Skeletal Muscle

Check water in cold room, need at least 28 liters for actin acetone powder, 32 liters for CapZ prep. (based on 1kg)
Put meat grinder and stirrers in cold room.
Put 4 liters (500g muscle) Acetone in cold room.
Check supply of cheesecloth. Order Grade 40 mesh (24x20 threads/inch from Fisher). Be careful if you borrow some that it is the correct mesh size! Chickens have been wasted!

All quantities are based on starting with 1kg of muscle.

1) Make 2 liters of Myosin extracting solution with cold water. Do not need to pH this solution.
   
   $0.5\text{ M KCl} \quad 74.55\text{ g}
   
   $0.1\text{ M K}_2\text{HPO}_4 \quad 34.85\text{ g} \quad \text{(THIS IS NOT MONOBASIC)}
   
   total \quad 2\text{ liters dd H}_2\text{O}
   
   (make before getting meat, or day before prep)

2) Use (~500g of Rabbits muscle) from the local abattoir, put directly into ice when received, and do the rest of the procedure in the cold room.

3) Grind muscle 1 times through meat grinder and weigh using student balance. Minimize time between grinding and extracting solution.

5) Place meat into 2 liter beakers, with 2 liters of cold myosin extracting solution. Stir with an overhead stirrer for 15 min.

6) Add 2 liters (less if doesn’t fit) of cold dd H2O. Stir 5 min.

7) Strain through 4 layers of cheesecloth using large funnel. (Do not skimp on size as the piece never seems to be big enough, I like 3ft x 3ft. It is OK to reuse the same piece)

8) Suspend meat in 3 liters (less if necessary) cold dd H2O. Stir 5 min. Strain through cheesecloth as above.

9) Suspend meat in 3 liters of cold 0.4% NaHCO3 (need 6l total). Stir 15 min. Strain through cheesecloth as above.

10) Repeat #9

11) Pour 2 liters cold dd H2O over muscle in funnel, and strain.
12) Resuspend meat in 2 liters of cold acetone. Do this and the following procedures using acetone in the fume hood. Stir 5 min.
    Strain through cheesecloth. Put waste acetone into sink diluted with lots of water.

13) Repeat #12. Strain well to remove as much acetone as possible, speeds drying.

14) Air dry meat over a large sheet of filter paper, in fume hood, with blower on for maximum airflow over meat. If convenient, can “stir up” muscle after a couple hours to speed up drying. Make gel sample of extracted muscle. Weigh acetone powder.

15) Store dry acetone powder in seal-a-meal bag at -70°C