

Preparation of Muscle Acetone Powder

Materials

1. 0.5 M KCl, 0.1 M K_2HPO_4 , pH 7.0, 4°C, 6 liters (extraction solution).
2. Meat grinder, set up in cold room.
3. Plastic pan for weighing muscle.
4. glassware : 4 liter beakers, stirring rods (or glass pipets), GSA bottles, 2 liter side-arm flask, large Buchner funnel with plurostopper, stirring bars and plates.
5. GSA rotors chilled in Sorvall centrifuges, may need two centrifuges.
6. Cold distilled water, one carboy.
7. 1 M Na_2CO_3 , 4°C, 100 ml.
8. Reagent-grade acetone, 12 liters at 4°C and 12 liters at room temperature.
9. Whatman #1 filter paper to match the funnel and also large ones for drying the powder.

Procedure (steps 1-9 at 4°C)

1. Sacrifice rabbit and dissect muscles (described elsewhere).
2. Grind muscles twice in a meat grinder in the cold room, use fine mesh for at least the second grinding. Weigh (typically 300-400 g per rabbit).
3. Extract for exactly 10 min with 3 volumes of extraction solution in a 4 liter beaker. Stir constantly with a rod or pipet.
4. Spin down muscle residues in a GSA rotor for 10 min at 12,000 rpm.
5. Collect pellets, repeat extraction as in step 3.
6. Spin down residues as in step 4.
7. Collect pellets in a 4 liter beaker. Add 3 volumes (measured as in step 3) of cold distilled H_2O and adjust pH to 8.2 to 8.5 with Na_2CO_3 (stir with rod or pipet).
8. Spin down residues as in step 4.

9. Repeat steps 7 and 8 until pellets begin to swell and edges start to appear semi-transparent (about 3-4 washes). Do not overwash.

10. Collect residues in several large beakers, add a total of 20 volumes or 12 liters cold acetone. Stir vigorously for 20 min in a fume hood. Manual stirring with a rod or pipet may be necessary.

11. Let most residues settle. Decant most acetone, filter out the remaining acetone with a Buchner funnel and Whatman #1 paper.

12. Repeat steps 10 and 11 with room temperature acetone.

13. Spread residues on large filter paper. Cover with aluminum foil and allow to dry overnight in the hood. Poke holes on foil so acetone can evaporate.

14. Weigh out dried powder. Expect about 40 g powder from 400 g tissue. Put ~10 g in each zip-lock bag and store dessicated in -70°C freezer.
