

PURIFICATION OF MUSCLE ACTIN I. PREPARATION OF ACETONE POWDER

Number:

Date:

BUFFERS:

1. KCl buffer:

Check:

Prepare 0.15 M K-phosphate buffer (pH 6.5):	Vol.: 1 l each	
0.15 M KH_2PO_4	20.414 g	_____
0.15 M K_2HPO_4	26.127 g	_____
Prepare KCl buffer:	Vol.: 1 l	
0.1 M KCl	7.456 g	_____

2. NaHCO_3 buffer: Vol.: 2 l

0.05 M NaHCO_3	8.401 g	_____
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3. EDTA:

Vol.: 1 l

1 mM EDTA (pH 7.0)	0.292 g	_____
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Refrigerate all buffers!!

Also needed: about 5 liters of chilled ddH₂O!

STEPS:

1. Preparation of Muscle Mince

- a. Euthanize rabbit (white male New Zealand, 7-8kg):

*Rabbit anaesthesia:

Rabbit weight:	Volume of added anaesthetics
35 mg/kg Ketamin	
7.5 mg/kg Xylozine	
0.02 mg/kg Atropin	

- b. Bleed of the rabbit completely
 c. Excise dorsal lateral skeletal and hind leg muscles (cca.350g)
 d. Chill muscle on ice (~30 min)
 e. Rinse muscle with distilled water (4°C)
 f. Mince/grind in prechilled, EDTA-washed meat grinder (4°C).

2. Extraction with KCl

- a. Quick extraction with stirring for 10 min in 1 liter of ice-cold KCl buffer
 b. Filtering through four layers of gauze (boiled in distilled water for 20 min, drained, and brought to 4°C; alternatively, use sterilized gauze)

3. Extraction with NaHCO_3 .

- a. Muscle mince extracted with stirring for 10 min

at 4°C in 2 liters of NaHCO₃

b. Filtering

4. Extraction with EDTA _____

a. Residue extracted with 1 liter of EDTA,
stirring for 10 min at 4°C

b. Filtration

5. Extraction with H₂O (2x!) _____

a. Residue extracted with 2 liters of distilled water,
stirring for 5 min at 4°C

b. Filtration

6. Extraction with acetone (5x!) _____

a. Residue extracted with 1 liter of acetone for 10 min,
stirring at 20-25°C

b. Filtration

7. Drying _____

a. Residue dried overnight in large glass evaporating dishes
(Typically, place large filter papers under the hood, and spread muscle extract on these. Cover muscle extract with dry gauze to avoid dust contamination.)

b. Storage of "acetone powder:"

Measure weight and overall yield.

Place powder in well closed glass vials.

Place vials in sandwich bags with desiccant added (drierite, CaSO₄).

Store at -80°C (for months).

8. Quantity of acetone-extracted muscle powder: