Preparation of myofibrils from glycerinated psoas muscle

Knight, P.J. & Trinick, J.A., (1982) Methods Enzymol. 85: 9

Number:

Buffers:

Rigor buffer:

	Vol: 1000 ml
0.1 *M* KCl	7.456 g
2 m*M* $MgCl_2$	2 ml stock (1 M)
1 m*M* EGTA	2 ml stok (0.5 M)
0.5 m*M* DTT (fresh)	0. 5 ml stock (1 M)
10 m*M* K-phosphate pH 7 (at 0°C)	prepare from concentrated stock

Na-Rigor buffer:

	Vol: 1000 ml
0.1 *M* NaCl	5.844 g
2 m*M* $MgCl_2$	2 ml stock (1 M)
1 m*M* EGTA	2 ml stok (0.5 M)
0.5 m*M* DTT (fresh)	0. 5 ml stock (1 M)
10 m*M* K-phosphate pH 7 (at 0°C)	prepare from concentrated stock

Steps:

1. Tease muscle into fine fiber bundles
2. Soak bundles in rigor buffer for 1 hr (Rotorack in 15 ml Falcon tube)
   Approximate muscle volume= ml
   Volume of added rigor buffer
3. Homogenize in 10 vol. rigor buffer (Ultra-Turrax on ice,
   in 50 ml Falcon tube)
4. Sediment at 2000*g* for 5 min. (3000 rpm, Janetzky large rotor, 4 °C)
   in 15 ml Falcon tube
5. Wash pellet in 10 vol. rigor buffer
6. Repeat steps 4-5 two to three times

Measuring protein concentration:

1. Centrifuge an aliquot of myofibrils and resuspend the pellet in Na-rigor buffer.
2. Dissolve suspension in warm 1% SDS.
3. Measure absorbace at 280 nm. Extinction coefficient: 0.7 ml/mg-cm.

Storage:

1. Sediment myofibrils from suspension by centrifugation.
2. Resuspend pellet with rigor buffer containing 50% glycerol.
3. Store at -20°C.