

Preparation of glycerinated psoas muscle**I. Buffers:****A. Rigor I.**

20mM Tris-HCl (pH 7.0)
 50mM KCl
 2mM EDTA
 4 mM MgCl₂
FRESH:
 5mM β-mercaptoethanol
 0.1 mM PMSF
 20 μg/ml Leupeptin
 10 μM E-64

Volume:

200 ml
 0.484 g
 0.746 g
 0.8 ml stock (0.5 M, pH 7)
 0.8 ml stock (1 M)

 78 μl
 0.1 ml stock (0.2 M in EtOH)
 100 μl stock (20 mg/ml)
 100 μl stock (10 mM)

B. Triton-Rigor I.

1 % Triton X-100
 Rigor I.

100 ml
 10 ml stock (10 %)
 to 100 ml

C. Rigor II.

10mM Tris-HCl (pH 7.0)
 75 mM KCl
 2 mM MgCl₂
 2mM EGTA
FRESH:
 1mM DTT (dithiothreitol)
 20 μg/ml Leupeptin
 10 μM E-64

100 ml
 0.121 g
 0.56 g
 0.2 ml stock (1 M)
 0.8 ml stock (0.25 M, pH 7)

 100 μl stock (1 M)
 100 μl stock (20 mg/ml)
 100 μl stock (10 mM)

D. 50 % glycerol-Rigor II.

10mM Tris-HCl (pH 7.0)
 75 mM KCl
 2 mM MgCl₂
 2mM EGTA
 50 % glycerol
FRESH:
 1mM DTT (dithiothreitol)
 20 μg/ml Leupeptin
 10 μM E-64

100 ml
 0.121 g
 0.56 g
 0.2 ml stock (1 M)
 0.8 ml stock (0.25 M, pH 7)
 50 ml (99.9 %, Sigma or Analar)

 100 μl stock (1 M)
 100 μl stock (20 mg/ml)
 100 μl stock (10 mM)

E. Triton X-100 stock solution

10 % Triton X-100
 ddH₂O

100 ml
 10 ml
 to 100 ml

Steps:

Do every step on ice!!

Soaking steps are on Rotorack, at 4°C (cold room)!!

1. Kill and bleed rabbit

2. Approach psoas muscle:

Quickly open peritoneal cavity. Push intestines on one side. Prepare under kidney. Expose psoas muscle.

3. Preparation of psoas strips:

Gently separate a bundle of fibers from the rest of the psoas muscle. Pull surgical thread underneath the bundle. Place a glass rod (or wooden stick) on the bundle. Tie the fiber bundle to the rod. Cut off bundle, and immediately transfer to Triton-Rigor I. solution. Repeat step 3 as required.

(Detergent treatment of muscle fibers:)

4. Soak fiber bundles in Triton-Rigor I. for 1 h.

5. Soak fiber bundles in fresh Triton-Rigor I. for 2 hrs.

6. Soak fiber bundles in fresh Triton-Rigor I. overnight

(Washing the detergent out:)

7. Soak in Rigor I. for 1 hr. Repeat 3x.

8. Soak in Rigor II. for 1 hr. Repeat 3x.

(Glycerination:)

9. Soak in 50 % glycerol solution for 1 hr. Repeat 2x.

10. Add fresh 50 % glycerol solution. Store at -20 °C.