

Myomesin Preparation

(Fürst, D.O., Vinkemeier, U., Weber, K. *J. Cell Sci.* **102**, 769, 1992;
Obermann, M.J., Plessmann, U., Weber, K., Fürst, D.O. *Eur. J. Biochem.* **233**, 110, 1995)

Number: Myomesin-2/96

I. Buffers:

A. LSB buffer (Low Salt Buffer, for myofibril washing) ——— with pyrophosphate

Volume: 500 ml

100 mM KCl	3.728 g
2 mM MgCl ₂	1 ml stock (1 M)
5 mM EGTA	5 ml stock (0.5 M)
1 mM β-mercaptoethanol	39 μl
1 mM NaN ₃	325 μl stock (1.54 M, 10 %)
10 mM Tris-maleate (pH 6.8)	0.6 g
2 mM Na ₂ P ₂ O ₇	0.446 g

Add fresh:

1 mM PMSF	2.5 ml stock (0.2 M in ethanol)
20 μg/ml Trypsin inhibitor	10 mg
10 μM E-54	500 μl stock (10 mg/ml)

B. LSB buffer (Low Salt Buffer, for myofibril washing) ——— without pyrophosphate

Volume: 2000 ml

100 mM KCl	14.912 g	1000 ml
2 mM MgCl ₂	4 ml stock (1 M)	7.456 g
5 mM EGTA	20 ml stock (0.5 M)	2 ml
1 mM β-mercaptoethanol	156 μl	10 ml
1 mM NaN ₃	1.3 ml stock (1.54 M, 10 %)	78 μl
10 mM Tris-maleate (pH 6.8)	2.42 g	0.65 ml
		1.21 g

Add fresh:

1 mM PMSF	10 ml stock (0.2 M in ethanol)	5 ml
20 μg/ml Trypsin inhibitor	40 mg	20 mg
10 μM E-54	2 ml stock (10 mg/ml)	

C. Extraction solution (high IS buffer for protein extraction): ———

Volume: 500 ml

0.6 M KCl	22.368 g
2 mM MgCl ₂	1 ml stock (1 M)
2 mM EGTA	2 ml stock (0.5 M)
1 mM β-mercaptoethanol	39 μl
10 mM imidazole-HCl (pH 7.0)	5 ml stock (1 M)

Add fresh:

1 mM PMSF	2.5 ml stock (0.2 M in ethanol)
20 μg/ml Trypsin inhibitor	10 mg

10 μ M E-54

500 μ l stock (10 mg/ml)

D. Buffer T with KCl (initial dialysis buffer):

Volume: 2000 ml

90 mM KCl	13.42 g
2 mM EGTA	8 ml stock (0.5 M)
0.3 mM DTT	92.5 mg
1 mM NaN ₃	1.3 ml stock (1.54 M, 10 %)
50 mM Tris-HCl (pH 7.9)	12.114 g

E. Buffer T (secondary dialysis buffer):

Volume: 2000 ml

2 mM EGTA	8 ml stock (0.5 M)
0.3 mM DTT	92.5 mg
1 mM NaN ₃	1.3 ml stock (1.54 M, 10 %)
50 mM Tris-HCl (pH 7.9)	12.114 g

F. 10x Buffer C (for DE52 equilibration):

Volume: 2000 ml

10 mM EDTA	40 ml stock (0.5 M)
3 mM DTT	0.9252 g
500 mM Tris-HCl (pH 7.9)	121.14 g

G. 1x Buffer C (for DE52 chromatography):

Mix 100 ml of 10x Buffer 'C' with 900 ml water.

H. 300 mM KCl Buffer C: (for gradient elution):

Volume: 500 ml

300 mM KCl	11.184 g
1x Buffer 'C'	to 500 ml

II. Steps:

Check:

A. Muscle Harvesting:

1. Optional muscles to be used:

- turkey breast
- chicken breast
- rabbit back

2. Quickly remove muscles

3. Cut muscles into small pieces

4. Quick-freeze muscles in liquid nitrogen

5. Store muscle at -80 °C.

B. Myomesin preparation:

1. Use about 100 g of frozen muscle _____
Weight of muscle used: _____
2. Quickly thaw muscle into LSB buffer (on ice) _____
Buffer Volume (3 ml/g-muscle): _____
3. Homogenize muscle for 2x30 s, on ice. _____
4. Centrifuge to collect myofibrils (3,000 g, 15 min, 4°C) _____
(Beckman etc. _____)
5. Wash myofibrils with LSB buffer three (3) times: _____
-resuspend in 2 ml/g-muscle LSB
Volume of LSB: _____
-centrifuge with 3,000 g, 15 min _____
6. Final pellet resuspended in Extraction buffer (3 ml/g-muscle) _____
Volume of Extracting Buffer: _____
7. Extract muscle for 35 min on ice. _____
8. Centrifuge (20,000 g, 50 min. 4°C) _____
9. Supernatant dialyzed against 90 mM KCl-Buffer T, for 3 hours at 4°C. _____
Volume of supernatant: _____
10. Supernatant dialyzed against Buffer T (no KCl) overnight at 4°C. _____
11. Centrifuge (185,000 g, 30 min, 4°C) _____
12. Add Ammonium Sulfate to supernatant to 40 % _____
Volume of supernatant: _____
Amount of (NH₄)₂SO₄ added: _____
13. Centrifuge (20,000 g, 30 min, 4°C) _____
14. Pellet dissolved in Buffer 'C' _____
Weight of pellet: _____
Volume of Buffer 'C' (try 1ml/g initially): _____
15. Dialyze against Buffer 'C': _____
16. Load sample onto DE52 column (equilibrated in Buffer 'C'): _____
17. Elute with 0-300 mM KCl linear gradient in a total volume of 350 ml. _____
Column dimensions (Typ 1.5x16 cm): _____
Chromatography Conditions:
a. Sample loaded onto column: _____
-Volume: _____
-OD: _____
b. Flow rate(Typ 1 ml/min): _____
c. Fraction size (Typ 4.4 ml): _____
d. Elution/fraction collection: _____

Pumped: YES / NO

- Void volume (Typ 15 ml):
- Paper speed: (Typ 6 cm/h):
- AUFS (Typ 0.1-0.2):

e. Elution profile:

Abs₂₈₀:

-UV-Monitor: attach records

-Spectrophotometer (260 nm, 280 nm): attach records

18. Gel electrophoresis:

Gel type:

Density:

Sample preparation:

Loading:

Running conditions: