DESMIN PURIFICATION

Based on Geisler, N. and Weber, K. Eur. J. Biochem. 111, 425-433, 1980.

Number:
Date:

Buffers:

1. Buffer A	2000 ml
40 mM imidazole-HCl (pH 6.9)	5.45 g
0.6 M KCl	89.46 g
1 mM EGTA	8 ml (0.25 M stock)
1 mM ß-mercaptoethanol (fresh)	140 μl

to 500 ml, add 0.5 % Triton X-100 (2.5 ml)

2. Extraction buffer	1000 ml
10 mM imidazole-HCl (pH 6.9)	0.681 g
0.6 M KI	99.6 g
1 mM EGTA	4 ml (0.25 M stock)
0.5 % Triton X-100	5 ml
0.5 nM DTT (fresh)	0.5 ml (1 M stock)
0.2 mM ATP (fresh)	0.67 ml (300 mM stock)

3. Urea Buffer	1000 ml	2000 ml
6 M urea	360.36 g	720.72 g
10 mM Na-phosphate (pH 7.5)	1.42 g	2.84 g
5 mM EGTA	20 ml	40 ml (0.25 M stock)
0.1 % ß-mercaptoethanol	1 ml	2 ml

For salt gradient:	
0.2 M KCl-Urea Buffer	100 ml
0.2 M KCl	1.49 g
Add Urea Buffer to 100 ml	

4. Tris-acetate buffer	1000 ml	2000 ml
0.01 M Trizma Base (pH 8.2 with acetic acid)	1.211 g	2.422 g
10 mM ß-mercaptoethanol	0.7 ml	1.4 ml

5. Sodium-bicarbonate buffer	1000 ml	2000 ml
1 mM $NaHCO_3$ (pH 8)	0.084 g	0.168 g
10 mM ß-mercaptoethanol	0.7 ml	1.4 ml

DEAE-cellulose (Whatman DE52): 16g swollen beads -> 50 ml volume

For ELFO, put aside ~100 μl samples, dissolve in ~5- μl 3x Laemmli solubilization buffer. If needed, add extra 1x Laemmli solubilization buffer (e.g., if there is substantial KDS precipitate.

Steps: Check:

1. Excise chicken gizzard (~20 g) _____

Weight of gizzard:

Set aside a small (~100 mg) piece; quick frees in LN2, store at –80˚C (for smitin ELFO)

2. Cut into small pieces _____

Homogenize in 12.5 ml/g-tissue Buffer A (with Triton),

stir 30 min on ice _____

4. Centrifuge, 9000 rpm, 10 min, GSA rotor _____

(Alternatively, spin in Janetzky 3500 rpm)

5. Resuspend **pellet** in 12.5 ml/g-tissue "Buffer A without Triton" _____

6. Stir 30 min on ice (ELFO #1) _____

7. Centrifuge, 9000 rpm, 10 min, GSA rotor _____

8. Repeat steps 5-7 _____

9. Resuspend **pellet** in 15 ml/g-tissue Extraction Buffer _____

10. Stir for 6 hours, 4 ˚C _____

11. Centrifuge, 9000 rpm, 10 min, GSA rotor

(Alternatively, Janetzky 3000 rpm, 4˚C, 10 min) _____

12. Repeat steps 9-11 2x (ELFO #2) _____

13. Resuspend pellet in 3.75 ml/g-tissue Urea Buffer _____

14. Stir at 37 ˚C for 2 hours to dissolve desmin _____

15. Centrifuge, 9000 rpm, 10 min, GSA rotor _____

16. Add 6-fold volume of ethanol to **supernatant** _____

Supernatant volume (ELFO #3):

Ethanol volume:

17. Centrifuge, 9000 rpm, 10 min, GSA rotor _____

17. Resuspend **pellet** in 1.25 ml/g-tissue Urea Buffer (ELFO #4) _____

18. Chromatography: Sephadex G-25 (500 ml) _____

Pump speed (Gilson): 600 (thin tubing)

AU Range: 0.1

Paper speed 1 mm/min

Approximate void volume: 600 ml

19. Chromatography: DEAE-cellulose (2.5 x 10 cm) (ELFO #5) _____

Pump speed (Gilson): 400

paper speed 0.5 mm/min

AU range: 0.05

Elute with 200 ml salt gradient (0-0.2 M KCl in Urea buffer) _____

Gradient mixer: 2 x 100 beakers connnected with tubing.

20. Screen fractions for desmin (OD, SDS-PAGE) _____

21. Pool desmin fractions (ELFO #6) _____

22. Dialyze against Tris-acetate buffer _____

23. Store (quick-freez in LN_2, store at –80 ˚C) _____