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Yeast Resource Center

http://depts.washington.edu/~yeastrc/ms_tap1.htm

Tandem Affinity Purification (TAP) Protocol

Modified from Rigaut et al. 1999.*

Stock solutions

100% NP-40	5 M NaCl	1 M Mg Acetate
100 mM DTT	1 M Tris-HCl, pH 8.0	100 mM EGTA, pH 8.0
1 M Imidazole	1 M $CaCl_2$	500 mM Na_2HPO_4
14.3 M β-mercaptoethanol	500 mM EDTA	500 mM NaH_2PO_4

BUFFERS

Note: For all buffers, add reducing agents immediately before use! For some complexes it is beneficial to add DTT to IPP300, IPP150 and NP-40 buffers.

IPP300 (100 mL)
Add protease inhibitors (page 3) and DTT.

Final Concentration	*Volume*	*Stock*
25 mM Tris-HCl, pH 8.0	2.5 mL	1 M
300 mM NaCl	6.0 mL	5 M
0.1% NP-40	1 mL	10%
Milli-Q water to 100 mL		

IPP150 (100 mL)
Add protease inhibitors (page 3) and DTT.

Final Concentration	*Volume*	*Stock*
25 mM Tris-HCl, pH 8.0	2.5 mL	1 M
150 mM NaCl	3.0 mL	5 M
0.1% NP-40	1 mL	10%
Milli-Q water to 100 mL		

* Rigaut G, Shevchenko A, Rutz B, Wilm M, Mann M, Seraphin B. A generic protein purification method for protein complex characterization and proteome exploration. Nat Biotechnol 1999;17(10):1030-2.

TEV Cleavage Buffer (TEV CB) (100 mL)

Final Concentration	*Volume*	*Stock*
25 mM Tris-HCl pH 8.0	2.50 mL	1 M
150 mM NaCl	3.0 mL	5 M
0.1% NP-40	1 mL	10%
0.5 mM EDTA	100 µL	500 mM
1.0 mM DTT	1 mL	100 mM
Milli-Q water to 100 mL		

Calmodulin-Binding Buffer (CBB) (100 mL)

Final Concentration	*Volume*	*Stock*
25 mM Tris-HCl, pH 8.0	2.5 mL	1 M
150 mM NaCl	3.0 mL	5 M
1 mM Mg acetate	100 µL	1 M
1 mM Imidazole	100 µL	1 M
2 mM $CaCl_2$	200 µL	1 M
10 mM β-mercaptoethanol	69.6 µL	14.3 M
Split into two 50-mL aliquots.		
Add 500 µL 10% NP-40 to one aliquot to make 0.1% NP-40.		
Add 100 µL 10% NP-40 to the other to make 0.02% NP-40.		

Calmodulin Elution Buffer (CEB) (20 mL)

Final Concentration	*Volume*	*Stock*
25 mM Tris-HCl, pH 8.0	0.50 mL	1 M
150 mM NaCl	0.60 mL	5 M
0.02% NP-40	40 µL	10%
1 mM Mg acetate	20 µL	1M
1 mM Imidazole	20 µL	1 M
20 mM EGTA	4 mL	100 mM
10 mM β-mercaptoethanol	14 µL	14.3 M
Milli-Q water to 20 mL		

NP-40 Buffer (700 mL)
Add protease inhibitors (page 3) and 1.0 mM DTT

Final Concentration	*Volume*	*Stock*
15 mM Na_2HPO_4	21 mL	500 mM
10 mM NaH_2PO_4-H_2O	14 mL	500 mM
1.0% NP-40	7 mL	100%
150 mM NaCl	21 mL	5 M
2 mM EDTA	2.8 mL	0.5 M
50 mM NaF	2.31 g	
0.1 mM Na_3VO_4	0.7 mL	100 mM

Immediately before use, add inhibitors and adjust to pH 7.2 with NaOH.

Protease Inhibitor	*Stock*	*Volume*
PMSF	0.1 M in 100% isopropanol	1 mL per 100 mL buffer
Benzamidine	0.5 M in 100% water	260 µL per 100 mL buffer
Pepstatin	10 mg/mL in HAc	100 µL per 100 mL buffer
Chymostatin	5 mg/mL in HAc	200 µL per 100 mL buffer
Aprotinin	10 mg/mL in water	100 µL per 100 mL buffer
Leupeptin	10 mg/mL in water	100 µL per 100 mL buffer

Procedure

1. Grow 2 L of yeast to 150 Klett units (4.5×10^7 cell/mL).

2. Pellet cells at 13,300 *g* for 5 min in Beckman Avanti J-25 centrifuge using the JA-9.1000 rotor and the 1 L centrifuge bottles.

3. Wash 1× with NP-40 buffer while transferring to 40 mL centrifuge tubes.

4. Pellet cells at 5000 *g* for 5 min in the JA 25.50 rotor.

5. Resuspend the cells in about 10–15 mL of NP-40 buffer and transfer to the 50 mL chamber of the bead beater.* Add glass beads† to about half to two-thirds full. Fill almost to the brim with NP-40 buffer. Attach cap and limit the amount of air trapped inside. Attach the outer chamber, place in an ice bath, and lyse cell 1 min on/1 min off, 10×, with a 5-min "off" period half-way through. Refill outer chamber with ice every third round.

*BioSpec Products, Inc., cat. no. 1107900.
† 425–600 µm. Wash with nitric acid before using; rinse until pH >6.0.

6. Check lysis by microscope. If <90%, do a few additional rounds.

7. Transfer lysate to 40 mL plastic centrifuge tube. Clarify at 5000 *g* for 10 min in a JA 25.50-rotor Beckman Avanti J-25 centrifuge.

8. Transfer crude lysate to tubes fitting the Type 45-Ti ultracentrifuge rotor. On a scale, adjust volume such that it almost fills the tube. Make sure opposite tubes are well balanced.

9. Spin lysates at 142,000 *g* for 1.4 hr at 4°C in a Beckman ultracentrifuge.

10. Retrieve the supernatant to a 50 mL Falcon tube. (Avoid disturbing the pellet and avoid collecting the top lipid layer.)

11. Add 1 mL of Sepharose 6B (Sigma) beads prepared in a 1:1 slurry with NP-40 buffer. Incubate on a rotating platform at 4°C for 30 minutes. Spin in a tabletop centrifuge to pellet the beads. Retrieve the supernatant into a new 50 mL Falcon tube.

12. Adjust NaCl concentration to 300 mM with 5 M NaCl.

13. Add 500 µL of IgG Sepharose 6 Fast Flow (Amersham)(previously prepared in a 1:1 slurry with NP-40 buffer) and incubate on a rotating platform at 4°C for at least 2 hr.

14. Pour the lysate/IgG Sepharose suspension onto a chromatography column (BioRad Poly-Prep) with a reservoir. Pull through with the pump.

15. Wash beads 2× with 10 mL of IPP300 buffer and 1× with 10 mL IPP150 buffer.

16. Wash beads with 10 mL of TEV CB.

17. Close the bottom with a stopper and add 1 mL of TEV CB and 5 µg of TEV. Plug the top of the column and incubate on a rotating platform between 2 hr and overnight at 4°C.

18. Drain eluate into a new column sealed at the bottom.

19. Wash out old column with 1.0 mL of TEV CB.

20. Add 3 vol (6 mL) of CBB to the TEV supernatant. Plus 3 µL of 1 M CaCl2 per mL of IgG eluate (~6 µL). Add 300 µL of calmodulin Sepharose 4B (Amersham) in CBB (1:1 slurry) and incubate on platform for 1 hr at 4°C.

21. Wash:
    - Wash 2× in 1 mL CBB (0.1% NP-40).
    - Wash 1× in 1 mL CBB (0.02 % NP-40).

22. Plug the bottom of the column and add 1 mL of CEB.

23. Elute first 1 mL fraction into a siliconized microfuge tube.

24. Plug the bottom of the column and add 1 mL of CEB.

25. Elute second 1 mL fraction into a siliconized microfuge tube.

26. Combine the fractions and split into 500 µL, 750 µL, and 750 µL into non-siliconized microfuge tubes.

27. Adjust aliquots to 25% TCA with 100% TCA and place on ice for 30 min with periodic vortexing.

28. Spin at maximum speed (13,000 rpm) in a microfuge at 4°C for 30 min.

29. Wash 1× with ice cold (-20°C) acetone containing 0.05 N HCl, and spin 5 min at maximum speed (13,000 rpm) at 4°C.

30. Wash 1× with ice cold (-20°C) acetone, and spin 5 min at maximum speed at 4°C.

31. Remove supernatant and dry in a speed vacuum for ~10 min.

Use the 500-µL fraction for silver stain on a 10% gel.
Send one 750-µL fraction for mass spectrometry.
Save one 750-µL fraction just in case.