



## 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 <sub>2</sub>	500 mM Na <sub>2</sub> HPO <sub>4</sub>
14.3 M β-mercaptoethanol	500 mM EDTA	500 mM NaH <sub>2</sub> PO <sub>4</sub>

### BUFFERS (for 2 liters of cells, scale up as needed)

*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 (25 mL)

Add protease inhibitors (page 3) and DTT.

Final Concentration	Volume	Stock
25 mM Tris-HCl, pH 8.0	625 μL	1 M
300 mM NaCl	1.5 mL	5 M
0.1% NP-40	250 μL	10%
Milli-Q water to 25 mL		

#### IPP150 (10 mL)

Add protease inhibitors (page 3) and DTT.

Final Concentration	Volume	Stock
25 mM Tris-HCl, pH 8.0	250 μL	1 M
150 mM NaCl	300 μL	5 M
0.1% NP-40	100 μL	10%
Milli-Q water to 10 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) (15 mL)**

<i>Final Concentration</i>	<i>Volume</i>	<i>Stock</i>
25 mM Tris-HCl pH 8.0	375 µL	1 M
150 mM NaCl	450 µL	5 M
0.1% NP-40	150 µL	10%
0.5 mM EDTA	15 µL	500 mM
1.0 mM DTT	150 µL	100 mM
Milli-Q water to 15 mL		

**Calmodulin-Binding Buffer (CBB) (10 mL)**

<i>Final Concentration</i>	<i>Volume</i>	<i>Stock</i>
25 mM Tris-HCl, pH 8.0	250 µL	1 M
150 mM NaCl	300 µL	5 M
1 mM Mg acetate	10 µL	1 M
1 mM Imidazole	10 µL	1 M
2 mM CaCl <sub>2</sub>	20 µL	1 M
10 mM β-mercaptoethanol	7 µL	14.3 M
Add Milli-Q water to 10 mL. Split into 9 mL and 1 mL aliquots.		
Add 90 µL 10% NP-40 to the 9 mL aliquot to make 0.1% NP-40.		
Add 2 µL 10% NP-40 to the 1 mL aliquote to make 0.02% NP-40.		

**Calmodulin Elution Buffer (CEB) (20 mL)**

<i>Final Concentration</i>	<i>Volume</i>	<i>Stock</i>
25 mM Tris-HCl, pH 8.0	62.5 µL	1 M
150 mM NaCl	75 µL	5 M
0.02% NP-40	5 µL	10%
1 mM Mg acetate	2.5 µL	1M
1 mM Imidazole	2.5 µL	1 M
20 mM EGTA	500 µL	100 mM
10 mM β-mercaptoethanol	1.8 µL	14.3 M
Milli-Q water to 2.5 mL		

**NP-40 Buffer (150 mL)**

Add protease inhibitors (page 3) and 1.0 mM DTT

<i>Final Concentration</i>	<i>Volume</i>	<i>Stock</i>
15 mM Na <sub>2</sub> HPO <sub>4</sub>	4.5 mL	500 mM
10 mM NaH <sub>2</sub> PO <sub>4</sub> -H <sub>2</sub> O	3 mL	500 mM
1.0% NP-40	1.5 mL	100%
150 mM NaCl	4.5 mL	5 M
2 mM EDTA	.6 mL	0.5 M
50 mM NaF	.5 g	
0.1 mM Na <sub>3</sub> VO <sub>4</sub>	150 µL	100 mM

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

<i>Protease Inhibitor</i>	<i>Stock</i>	<i>Volume</i>
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 DMSO	100 µL per 100 mL buffer
Chymostatin	5 mg/mL in DMSO	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 ( $4.5 \times 10^7$  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 (or equivalent).
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 or equivalent.
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. Pop air bubbles using a pastetur pipette and bulb. Attach cap and limit the amount of air trapped inside. Attach the outer chamber, place in an ice bath, and lyse cells 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 in a 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. Balance tubes.
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 hard 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. This is a clearing step.
12. Adjust NaCl concentration to 300 mM with 5 M NaCl. This is a good stringency to start with.
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 CaCl<sub>2</sub> 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  $\mu$ L, 750  $\mu$ L, and 750  $\mu$ L 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 $\times$  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 $\times$  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- $\mu$ L fraction for silver stain on a 10% gel.

Send one 750- $\mu$ L fraction for mass spectrometry.

Save one 750- $\mu$ L fraction just in case.

To ensure optimal results for your purification follow these handy tips.

- Check by PCR the presence of the TAP tag on your protein of interest.
- Do a small prep to make sure your protein is in the soluble fraction.
- Check health of cells with the TAP tag by doing a growth curve. A healthy haploid strain doubles between 80-90 minutes.
- Wear gloves to protect your samples from keratin, which can show up in mass spec.
- Test activity of TEV protease to ensure good cleavage off the IgG column.  
Commercial TEV preparations do not always work well.
- Make sure samples are kept cold throughout the purification, especially during lysis.