Protocol for TAP Tag Purification

Tandem Affinity Purification (TAP) Reference:
Rigaut, G., Shevchenko, A., Rutz, B., Wilm, M., Mann, M., and Seraphin, B. 1999. A generic protein purification method for protein complex characterization and proteome exploration. *Nature Biotechnology* **17**: 1030-1032. The following protocol for *S. pombe* was adapted from the Seraphin Lab protocol. web site: http://www.embl-heidelberg.de/ExternalInfo/seraphin/TAP.html

Stock Solutions:

10% NP-40	5M NaCl	0.5 M EDTA
1 M DTT	1 M Tris-HCl pH 8.0	1 M Mg^{2+}Acetate
1 M Imidazole	1M $CaCl_2$	0.5 M EGTA

14.3 M β-Mercaptoethanol (BME)

Buffers:

IPP150 (100 ml)

[Final]			[Stock]
10 mM	Tris-HCl pH 8.0	1 ml	1 M
150 mM	NaCl	3 ml	5 M
0.1%	NP-40	1 ml	10%
	↑ volume to 100 ml		

TEV Cleavage Buffer (TEV CB) (50 ml)

[Final]			[Stock]	
10 mM	Tris-HCl pH 8.0	0.5 ml	1 M	
150 mM	NaCl	1.5 ml	5 M	
0.1%	NP-40	0.5 ml	10%	
0.5 mM	EDTA	50 µl	0.5M	
1.0 mM	DTT	50 µl	1 M	*
	↑ volume to 50 ml			

IPP150 Calmodulin Binding Buffer (CBB) (50 ml)

[Final]			[Stock]	
10 mM	Tris-HCl pH 8.0	0.5 ml	1 M	
150 mM	NaCl	1.5 ml	5 M	
1 mM	Mg^{2+}Acetate	50 µl	1 M	
1 mM	Imidazole	50 µl	1 M	
2 mM	$CaCl_2$	100 µl	1 M	
10 mM	BME	34.95 µl	14.3 M	*
	↑ volume to 50 ml			

divide into two 50 ml aliquots
adjust one 50 ml aliquot to 0.1% NP-40 by adding 500 µl 10% NP-40
adjust one 10 ml aliquot to 0.02% NP-40 by adding 20 µl 10% NP-40
***Add DTT or BME immediately before use.**

TAP Tag Purification
Buffers (continued)

IPP150 Calmodulin Elution Buffer (CEB) (10 ml)

[Final]			[Stock]
10 mM	Tris-HCl pH 8.0	0.1 ml	1 M
150 mM	NaCl	0.3 ml	5 M
0.02%	NP-40	20 µl	10%
1 mM	Mg^{2+}Acetate	10 µl	1 M
1 mM	Imidazole	10 µl	1 M
20 mM	EGTA	400 µl	0.5 M
10 mM	BME	* 7.0 µl	14.3 M
	↑ volume to 10 ml		

Note: 0.05% NP-40 may be used in this buffer.
***Add DTT or BME immediately before use.**

NP-40 Buffer (3 L)

[Final]		[Stock]
6 mM Na_2HPO_4	2.56 g	
4 mM $NaH_2PO4{\cdot}H_20$	1.66g	
1% NONIDET P-40	30 ml	100%
150 mM NaCl	26.3 g	
2 mM EDTA	12 ml	0.5 M
50 mM NaF	6.3 g	
4 ug/ml leupeptin	12 mg	
0.1 mM Na_3VO_4	3 ml or 0.6 ml	100 mM 0.5 M
	↑ volume to 3 L	

Add the following protease inhibitors per 50 ml NP-40 buffer:
1 complete tablet, EDTA free (Boehringer Mannheim Cat. No. 1873580)
130 µl 0.5 M Benzamidine prepared fresh in 100% ethanol (400 mg in 5 ml EtOH)
500 µl 0.1 M PMSF prepared fresh in 100% ethanol (87 mg in 5 ml EtOH)

Materials:
Acid Washed Glass Beads (425-600 microns); Sigma Cat. No. G-8772
Bead Beater; BioPsec Products Cat.#1107900
50 ml tube Falcon 2070
Nalgene 25X89 mm tubes; Cat.# 3430-2526
Biorad Poly-Prep Chromatography Column 0.8 X 4 cm; Cat. # 731-1550
TEV; Gibco Cat. # 10127-017
Silver Stain Kit; Amersham Cat. # 17-1150-01
Novex Dry Ease Kit; Cat. # NI2387
IgG Sepharose; Pharmacia Cat.#17-0969-01
Calmodulin Affinity Resin; Stratagene Cat. #214303-52

Purification Procedure:

1. Grow 2L of yeast in 4X YE to 1.0 x 10^7 – 1.4 x 10^7 (OD_{595} = 0.5-0.7)

2. To prepare protein lysate, the yeast cells are lysed in a bead beater
   a. completely fill the lysis chamber with cell pellet and glass beads
   b. Add NP-40 buffer plus Protease Inhibitors until entire chamber is immersed then screw it into the ice-water jacket and add ice + water as heat sink.
   c. To lyse cells: 30 seconds on, pause 30 seconds to dissipate heat, repeat for ~7 minutes until sufficient lysis is achieved (~60-90% → take a small aliquot and check efficiency by microscopy)

3. Transfer lysate to 50 ml Falcon tube, extract beads with NP-40 plus inhibitors and transfer to 50 ml tube. Pellet for 3-5 min. in table-top centrifuge at 4°C and 3500 rpm.

4. Retrieve supernatant to a 50 ml Falcon tube (note: avoid collecting the bottom pellet and the top lipid layer). Add 1ml IgG Sepharose beads in NP-40 Buffer (1:1 slurry) and incubate on a rotating platform at 4°C for 1 hr.
Note: To prepare IgG/Sepharose, wash IgG/Sepharose Beads with NP-40 Buffer (no inhibitors) to remove ethanol, and resuspend in 1:1 slurry with NP-40 buffer. Want 500ul dry beads + 500ul NP-40 to make 1:1 slurry

5. Pour lysate and beads onto a Biorad Poly-Prep Chromatography Column (0.8 X 4 cm) with a reservoir. Allow the column to pack by gravity.

6. Wash beads with 30 ml IPP150 Buffer (3 times, 10ml each wash), make sure beads come up each time

7. Wash beads with 10 ml TEV cleavage buffer (prepared fresh)

8. Close bottom of the column with a stopper (supplied with column).
   Add 1 ml TEV cleavage buffer and 300-500 U TEV (50 ul).
   Plug top of column (plug supplied) and incubate on rotating platform for 1.5 hr at 16°C.

9. Drain eluate into a new column that is sealed at the bottom.
   Wash out old column with 1.0 ml TEV CB.

10. Add 3 volumes of C**B**B (6ml 0.1% CBB+13ul $CaCl_2$) to the TEV supernatant in the new column plus 3 µl 1M $CaCl_2$ per ml IgG eluate, add 300 µl Calmodulin resin in C**B**B (1:1 slurry), and incubate on rotating platform for 1 hr at 4°C. (Wash CBB beads beforehand with 0.1% NP-40 CBB buffer (3x 1ml)

11. Wash beads 1X 1 ml C**B**B (**0.1%** NP-40).
    Wash beads 1X 1 ml C**B**B (**0.02%** NP-40).
Note: detergent is harmful for mass spec.

12. Elute twice with 1.0 ml C**E**B containing 0.02% NP-40 into a 1.5 ml eppendorf on ice. Before putting into eppendorf, put cap on end of column, flick a couple of times and let sit for a couple of minutes.

13. Split eluate in half into 2 eppendorf tubes, and TCA precipitate proteins. 1st elution put 80% (800ul) into tube for mass spec. Put 20% (200ul) into tube for silver staining. 2nd elution put 80% (800ul) into tube for silver staining and 20% (200ul) into tube for mass spec. Add 333ul of 25% TCA to each tube to precipitate.

TCA precipitation: Adjust eluate to 25% TCA with 100% TCA and place on ice for 30' with periodic vortexing.

Spin max speed at 4°C for 30 minutes.

Wash 1X ice- cold (-20°C)(1ml) acetone containing 0.05 N HCl, spin 10 minute max speed 4°C.
Wash 1X ice-cold (-20°C)(1ml) acetone only, spin 10 minute max speed at 4°C.
Remove supernatant carefully, and dry pellets in the speed vac.

Pellet 1→ save for mass spec.

Pellet 2 → resuspend in 1X gel sample buffer (35ul LDS Sample Buffer), resolve on SDS-PAGE and Silver stain with Amersham kit .

Note: We prefer the use of 4-12% Novex gels with MOPS buffer at this step.