TAP-tag Based Purification of Yeast Proteins

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[Abstract] Tandem affinity purification (TAP) is used to look at protein-protein interaction. Its use relies on generating a fusion protein with a TAP tag on the C- or N- terminal end. In this protocol, a two-step purification of N-terminus TAP-tagged proteins from yeast is described.

Materials and Reagents

1. Complete Protease Inhibitor Cocktail Tablet (Roche Diagnostics)
2. 100% NP-40 (Sigma-Aldrich)
3. AcTEV Protease (Life Technologies, Invitrogen™)
4. IgG Sepharose 6 Fast Flow (GE Healthcare Life Sciences)
5. Calmodulin affinity resin (Guidechem/Stratagene)
6. SDS lysis buffer
7. beta-ME
8. Leupeptin
9. Mg_2 acetate
10. Acetone
11. BME
12. EDTA
13. $CaCl_2$
14. NaF
15. Imidazole
16. NP40 buffer (see Recipes)
17. IPP150 buffer (see Recipes)
18. IPP150 calmodulin binding buffer (see Recipes)
19. TEV cleavage buffer (see Recipes)
20. IPP150 calmodulin elution buffer (see Recipes)

Equipment

1. Avestin Homogenizer (Avestin®)
2. Beckman centrifuge and rotor (Beckman Coulter)
3. Polycarbonate tubes
4. Chromatography column
5. Microfuge tube
6. Shaker

Procedure

Day 1

1. Grow 4,000 OD's cells (two 2 L cultures in 8 L flasks, grow to 1 OD/ml) 30 °C shaker.
2. Split to four 1 L centrifuge bottles. Spin at 7,000 rpm for 10 min. While spinning, make 60 ml NP40 Buffer before beginning.
3. Wash sample in each bottle with 250 ml cold H_2O. Spin at 7,000 rpm for 10 min.
4. Resuspend all cells in 50 ml total volume cold NP-40 buffer (add 30 ml buffer to first bottle, vortex, transfer all to next bottle, and so on).
5. Lyse cells using Avestin Homogenizer (4 passes) – see instructions for usage before beginning.
6. Split lysate into three polycarbonate tubes for 70 Ti Rotor for high-speed spin (~15 ml in each + 1 blank – weigh out to assure accuracy).
7. Spin at 100,000 *x g* for 30 min (37,500 rpm in an ultracentrifuge).
8. Pour supernatants into a 50 ml conical.
9. Take 100 µl cleared lysate sample (100 µl of 50 ml sample = 1x).
10. Prep IgG Sepharose in microfuge tube.
    a. 1 ml of IgG sepharose slurry (500 µl beads in 1:1 slurry) in 15 ml tube.
    b. Wash 3x 5 NP-40 lysis buffer w/o PI's 2 min 4,000 rpm.
11. Add 1 ml cleared lysate directly to microfuge tube with beads and place beads into 50 ml conical with cleared lysate. Repeat once more with 1 ml more of lysate to obtain all of the beads.
12. Rotate 2 h in cold room.
13. Remove 1% of total sample (500 µl), wash 3x 14K for 30 sec with NP-40 buffer, resuspend in 100 µl SDS lysis buffer + 1x PI (IgG bound sample – this is 5x).
14. Pour samples over chromatography column in cold room (you will have to do this is stages because the column can only hold 10 ml at one time – keep samples cold while waiting).

If samples are draining very slowly, it is because nucleic acids are plugging up column so you can always transfer everything to new column to speed things up.

15. Take 100 μl IgG unbound sample from flowthrough (1x).
16. Wash column
    c. 3x 10 ml IPP150
    d. 1x 10 ml TEV Cleavage Buffer + DTT (after last wash, remove rest of TEV Buffer with a gel tip)
17. Resuspend sepharose in 1 ml TEV Cleavage Buffer + DTT
    e. Transfer to 1.5 ml Eppendorf.
    f. Add 300 units TEV protease (30 μl– located in -80 freezer).
    g. Rotate in cold room overnight.

Day 2

1. Spin down samples at 2,000 *x g* cold and place supernatant (1 ml) in 15 ml cold conical.
2. Resuspend sepharose again in 1 ml TEV Cleavage Buffer + DTT.
    a. Spin 2,000 *x g* cold, and add supernatant to same 15 ml conical (now you have a total of 2 ml).
3. Spin 15 ml conical at 5,000 rpm for 5 min to pellet excess beads. Transfer supernatant to new cold 15 ml conical.
4. Add 6 ml 0.1% Calmodulin binding buffer + BME + 6 μl 1 M $CaCl_2$.
5. Take 50 μl TEV cleaved sample (50 μl of 8 ml is 6.25x).
6. To obtain TEV uncleaved sample
    a. Add 1 ml TEV cleavage buffer to beads, spin 2,000 *x g*.
    b. Wash 3x in TEV cleavage buffer and transfer to new tube on last wash.
    c. Resuspend in 1ml TEV cleavage buffer (1.5 ml total).
    d. Remove 45 μl of beads (3% of total).
    e. Resuspended in 100 μl of SDS lysis buffer – 15x.
7. Preparation of calmodulin beads – use 500 μl bead (1 ml of a 1:1 slurry).
    a. Wash 3x 1 ml 0.1% calmodulin binding buffer 2 min 14 K in microfuge tube.
8. Add beads to samples in 15 ml conical as in Step 8 of Day 1.
    a. Bind samples in cold room 2 h.
9. Pour samples over chromatography column in cold room.
10. Take 100 μl calmodulin unbound sample (6.25x).
11. Wash column
    a. Wash 2x 1 ml 0.1% calmodulin binding buffer + BME.
    b. Wash 1x 1 ml 0.02% calmodulin binding buffer + BME.
12. Elute w/ 1 ml calmodulin elution buffer + BME (eluate 1) - collect in microfuge tube.

13. Add 700 microliters calmodulin elution buffer + BME.
    a. Keep in cold room for 10 min.
    b. Eluate into Eppendorf (eluate 2).
14. Take 20 μl of eluate 1 – 50x.
15. Take 14 μl of eluate 2 – 50x.

TCA precipitate eluates

1. Adjust eluates to 25% TCA w/ 100% TCA.
    a. 333 μl of TCA for 1 ml sample; 233 μl of TCA for 700 μl sample.
2. Place samples on ice 30 min w/ periodic vortexing.
3. Spin max cold for 10 min.
4. Rotate 180 degrees, spin at max speed in the cold for 30 min (since pellet will collect away from center of centrifuge, you want to aspirate from side of eppendorf facing inwards).
5. Wash 1x 1 ml cold acetone + 0.05 N HCl.
6. Spin max cold 5 min, rotate 180 degrees spin max cold 5 min.
7. Wash again w/ cold acetone.
8. Spin max cold 5 min.
9. Remove supernatant carefully, dry pellets.
10. Freeze pellets -80 °C.

Recipes

1. IPP150 buffer (100 ml)

2.5 ml 1 M Tris-HCl (pH 8)	25 mM Tris-HCl (pH 8)
3 ml 5 M NaCl	150 mM NaCl
1 ml 10% NP-40	0.1% NP-40

   Add H_2O to final volume.

2. TEV cleavage buffer (50 ml)

1.25 ml 1 M Tris-HCl (pH 8)	25 mM Tris-HCl (pH 8)
1.5 ml 5 M NaCl	150 mM NaCl
0.5 ml 10% NP-40	0.1% NP-40
50 ml 0.5 M EDTA	0.5 mM EDTA

   Add H_2O to final volume.
   Add 0.5 μl of 1 M DTT/ml before use (0.5 mM DTT).

3. IPP150 calmodulin binding buffer (100 ml) – for 2x buffer without detergent

2.5 ml 1 M Tris-HCl (pH 8) (25 mM)	5 ml

3 ml 5 M NaCl (150 mM) 6 ml

100 µl 1 M Mg_2 Acetate (1 mM) 200 µl

100 µl 1 M Imidazole (1 mM) 200 µl

200 µl 1 M $CaCl_2$ (2 mM) 400 µl

Add H_2O to final volume.

Divide into two 50 ml aliquots.

Adjust one 50 ml aliquot to 0.1% NP-40 by adding 500 µl 10% NP-40.

Adjust other 50 ml aliquot to 0.02% NP-40 by adding 100 µl 10% NP-40.

Add 0.7 µl of 100% (m/v) beta-ME per ml before use (10 mM beta-ME).

4. IPP150 calmodulin elution buffer (10 ml) – for 2x buffer without detergent

   0.25 ml 1 M Tris-HCl (pH 8) (25 mM) 500 µl

   0.3 ml 5 M NaCl (150 mM) 600 µl

   20 microliters 10% NP-40 (0.02%) 40 µl

   10 microliters 0.5 M Mg_2 Acetate (0.5 mM) 20 µl

   10 microliters 1 M Imidazole (1 mM) 20 µl

   400 microliters 0.5 M EGTA (20 mM) 800 µl

   Add H_2O to final volume.

   Add 0.7 µl of 100% (m/v) beta-ME per ml before use (10 mM beta-ME).

5. NP-40 Buffer (1 L) – for 2x buffer without detergent

   1.61 g $Na_2HPO_4*7H_2O$ (6 mM) 3.22 g

   0.553 g $NaH_2PO_4*H_2O$ (4 mM) 1.106 g

   100 ml 10% NP-40

   8.77 g NaCl (150 mM) 17.54 g

   4 ml 0.5 M EDTA (4 mM) 8 ml

   400 microliters leupeptin 800 µl

   2.1 g NaF (50 mM) 4.2 g

   0.0552 g Na_3VO_4 (300 µM) 0.11 g

   Add H_2O to final volume.

   Add the following protease inhibitors per 50 ml NP-40 buffer before use:

   1 complete tablet, EDTA free protease inhibitors (crush tablet first in weigh paper).

   100 microliters 0.5 M PMSF in DMSO.

6. Samples to take to monitor efficiency

   Cleared lysate, IgG bound, IgG unbound, TEV cleaved, TEV uncleaved, calmoldulin unbound, eluate (pre-TCA precipitation).

Acknowledgments

This protocol has been modified and adapted in the Espenshade Lab, Johns Hopkins School of Medicine. Funding to support different projects that have used this protocol has come from NIH – National Heart, Lung, and Blood Institute, National Institute of Allergy and Infectious Diseases, the Pancreatic Cancer Action Network, and the American Heart Association.

References

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