TAP-TAG Purification

Cell Lysis

1. Lyse cells in lysis buffer (add 2 mM DTT and 1X PIC). Use 1 mL lysis buffer per 150 mm plate (typically 10 x 150 mm plates of 293 cells per purification).
2. Freeze cell lysate in liquid nitrogen and rapidly thaw in a cool water bucket. Do this step twice.
3. Incubate on ice 30 min
4. Spin down lysate 10 min in microcentrifuge max speed 4ºC
5. Filter cleared supernatant using 0.45 µm filter

IgG-Sepharose Binding

6. Wash 100 µL beads (packed) 3x with lysis buffer. All bead spins at 1500 rpm 2 min 4ºC
7. Add cleared lysate to the washed IgG beads. Incubate 4 hrs rocking 4ºC

TEV Cleavage

8. Spin down beads
9. Wash beads 3x with 1 mL lysis buffer
10. Wash beads 3x with 1 mL TEV buffer (Add 1 mM DTT)
11. Resuspend beads in 300 µL TEV buffer containing 70 µL TEV protease
12. Incubate overnight at 4ºC rotating

Calmodulin-Sepharose Binding

13. Wash 100 µL Calmodulin beads 3x in Calmodulin binding buffer (Add 10 mM BME)
14. Spin down IgG beads. Transfer supernatant from IgG beads to a new tube
15. Add 300 µL Calmodulin binding buffer to IgG beads. Invert to mix, spin down, collect supernatant. Repeat a total of three times (should have 1200 µL at end)
16. Add 1/250 volume (5 µL) of 1M $CaCl_2$. Mix by inversion.
17. Spin down to remove any residual IgG beads
18. Transfer supernatant to washed Calmodulin-sepharose beads.
19. Incubate 90 min at 4ºC rotating

Calmodulin-Sepharose Elution

20. Spin down beads and remove supernatant
21. Wash beads 3x with 1 mL Calmodulin <u>binding</u> buffer
22. Wash beads 3x with 1 mL Calmodulin <u>rinsing</u> buffer
23. Resuspend beads in 100 µL boiling 1X SB
24. Boil 10 min
25. Collect eluted proteins by centrifugation. Centrifuge again to remove residual beads.
26. Concentrate into 20 µL on YM-10 centricon at 4ºC
27. Run all 20 µL on precast 4-15% SDS-PAGE Gel
28. Rinse gel three times in ddH_20 for 5 min each at RT rocking
29. Stain gel in Peirce Gelcode Colloidial Coomaissie Blue for 1.5 hrs at RT rocking
30. Destain in ddH_20 for 1 hr at RT rocking then leave overnight at 4ºC

TAP-TAG Purification Buffers

Lysis Buffer
10% Glycerol
50 mM HEPES-KOH pH 7.5
100 mM KCl
2 mM EDTA
0.1 % NP-40
10 mM NaF
0.25 mM Na_3VO_4
50 mM β-glycerolphosphate
2 mM DTT – add at time of use
1 X Protease Inhibitor Cocktail – add at time of use

TEV Buffer
10 mM HEPES-KOH pH 8.0
150 mM NaCl
0.1 % NP-40
0.5 mM EDTA
1 mM DTT – add at time of use

Calmodulin-Binding Buffer
10 mM HEPES-KOH pH 8.0
150 mM NaCl
1 mM Mg Acetate
1 mM Imidazole
0.1 % NP-40
2 mM $CaCl_2$
10 mM B-mercaptoethanol – add at time of use (7.8 µL per 10 mL)

Calmodulin-Rinsing Buffer
50 mM Ammonium bicarbonate pH 8.0
75 mM NaCl
1 mM Mg Acetate
1 mM Imidazole
2 mM $CaCl_2$

Calmodulin-Elution Buffer (DO NOT USE)
50 mM Ammonium bicarbonate pH 8.0
25 mM EGTA