Tandem Affinity Purification (TAP) Protocol

Material: Rice suspension cultured cells

Protein Extract Preparation

1, Rice suspension cells were harvested in liquid nitorogen.

2, Frozen tissues were homogenized to a fine powder with a motor and pestile under evaporating liquid nitrogen.

3, The resultant powder was resuspended in 2 volumes of chilled TBSN (20mM sodium phosphate pH7.5, 300mM NaCl, 0.1% NP-40).

4, The mixture was filtered through 2 layers of Miracloth, centrifuged at 10,000g for 20min at 4°C, and filtered through a 0.45μm membrane (Millipore).

Purification and Elution of Protein

1, A 50mg portion of protein extract was diluted to 45ml in TBSN and incubated with 300μl IgG sepharose 6 fast flow (GE Healthcare) on a rotator at 4°C overnight.

2, After washing the beads 3 times with 10ml of TBSN, pour the beads to chromatography empty column (BioRad Poly Prep).

3, Wash the beads 3 times with 1ml of TEV cleavage buffer (50mM Tris-HCl, pH8.0, 0.5mM EDTA, 1mM DTT).

4, Close the bottom, and add 1ml of TEV cleavage buffer and 5μg of AcTEV protease (Invitrogen). The column was rotated at 4°C overnight.

5, Drain eluate into a new column sealed at the bottom.

6, Wash out old column with 1ml TEV cleavage buffer and mix eluate to the new column.

7, Add 3 volumn (6ml) of Calmodulin-binding buffer (25mM Tris-HCl, pH8.0, 150mM NaCl, 1mM Mg acetate, 1mM imidazole, 2mM $CaCl_2$, 10mM 2-Me) with 0.02% NP-40 and 6μl of 1M $CaCl_2$.

8, Add 300μl of calmodulin Sepharose 4B (GE Healthcare) and incubate on a rotary shaker for 1h at 4°C.

9, Wash twice with 1ml of Calmodulin-binding buffer with 0.1% NP-40.

10, Wash once with 1ml of Calmodulin-binding buffer with 0.02% NP-40.

11, Plug the bottom of the column and add 2ml of Calmodulin elution buffer (25mM Tris-HCl, pH8.0, 150mM NaCl, 0.02% NP-40, 1mM Mg acetate, 1mM imidazole, 20mM EGTA, 10mM 2-Me).