**CRTC2 TAD and TADL Purification**

Peptide: CRTC2 TAD (654-691+Y) and CRTC3 TAD long (601-691+Y)

Vector: pMCSG10

Competent cells: BL21 (DE3)

**GROWTH PROCEDURE**

Day 1

Transform competent cells. Incubate LB plate at 37 ºC overnight.

Day 2, morning

1. Using sterile serological pipet, add 50 mL TB plus ampicillin to a sterilized flask.
2. Inoculate the flash with a single colony.
3. Incubate with 225 rpm shaking at 37 ºC.
4. When A600 reaches 0.8 – 1.0, pour 50 ml culture into 1 L TB plus ampicillin.
5. Shake at 225 rpm, at 37 ºC and monitor cell growth by measuring A600.
6. When A600 reaches 1 – 1.2, lower temperature to 16 ºC and shake for 30 min.
7. Add 1 mL of stock IPTG and shake culture at 16 ºC overnight.
8. Harvest cells by centrifuging at 4 ºC for 30 minutes.
9. Store cell pellet at – 80 ºC freezer.

**PURIFICATION**

**Buffers**

* PBS buffer

140 mM NaCl, 2.7 mM KCl, 10 mM Na2HPO4, 1.8 mM KH2PO4, pH 7.3, 5 mM EDTA)

* Glutathione elute buffer

50 mL (50 mM Tris-HCl, 10 mM Reduced Glutathione, pH 8.0, 200 mM NaCl)

* Dialysis buffer

25 mM Tris, 200 mM NaCl, pH 8.0

Filter and cool buffers before use and perform purification procedure in cold room to prevent degradation.

**Procedure**

1. Resuspend cell pellet from 1 L culture in 80 mL of cold PBS buffer.
2. Add 800µL PMSF, 80µL pepstatin A, 80µL leupeptin, 800µL Triton-X 100. Vortex and then incubate at 4 ºC for 30 minutes.
3. Sonicate for 5 minutes with 1 second on, 5 seconds off, 5 output (program ID 1).
4. Centrifuged at 4 ºC, at 12000 RPM for 30 minutes.
5. Discard pellet and save soluble supernatant.
6. Incubate supernatant with 8 mL of PBS equilibrated glutathione sepharose resin.
7. Collect flow through.
8. Wash resin with 5 bed volumes (~ 40 ml) PBS buffer, collect wash 1.
9. Wash resin with 5 bed volumes (~ 40 ml) PBS buffer, collect wash 2.
10. Wash resin with 5 bed volumes (~ 40 ml) PBS buffer, collect wash 3.
11. Incubate resin with 15 ml elute buffer at room temperature for 15 minutes, and collect E1.
12. Incubate resin with 15 ml elute buffer at room temperature for 15 minutes, and collect E2.
13. Incubate resin with 15 ml elute buffer at room temperature for 15 minutes, and collect E3.
14. Run gel and combine fractions containing protein.
15. Dialyze against 1 L dialysis buffer at 4 ºC overnight. (Concentrate protein after this step if purify tagged protein).
16. Add 2 ml TEV and shake at room temperature for 4 hour and then 4 ºC overnight.
17. Prepare nickel resin.
18. Incubate TEV treated protein with nickel resin equilibrated with dialysis buffer at 4 ºC for 1 hour.
19. Collect flow through, filter (using 0.22 uM syringe filter) and inject to HPLC column.
20. Wash resin with 45 ml of dialysis buffer.
21. Collect wash, filter and inject to HPLC.

**Glutathione Sepharose 4B**

**Materials**

* 0.1 M Tris-HCl + 0.5 M NaCl, pH 8.5
* 0.1 M NaAcetate + 0.5 M NaCl pH 4.5
* 1X PBS (140 mM NaCl, 2.7 mM KCl, 10 mM Na2HPO4, 1.8 mM KH2PO4, pH 7.3, 1mM EDTA)
* 6 M Guanidine HCl

**Regeneration Procedure** *(from the Amersham procedure)*

1. Wash specifically bound proteins off beads
   1. Wash with 2 bed volumes 0.1 M Tris-HCl + 0.5 M NaCl, pH 8.5
   2. Wash with 2 bed volumes 0.1 M Na-Acetate + 0.5 M NaCl, pH 4.5
   3. Repeat 3times
   4. Re-equilibrate with 5 bed volumes H2O
2. Remove precipitated and denatured proteins
   1. Wash with 2 bed volumes 6 M guanidine HCl
   2. Immediately wash with 5 bed volumes H2O
3. Remove hydrophobic substances
   1. Wash with 3-4 bed volumes 70% ethanol (alternatively, use 2 bed volumes 0.1% non-ionic detergent)
   2. Immediately wash with 5 bed volumes 1X PBS
4. For long-term storage
   1. Wash 2x with 10 bed volumes 1X PBS
   2. Wash 2x with 10 bed volumes 20% ethanol
   3. Store at 4°C