CREB bZip

CREB bZip(C300S)

CREB bZip(C300S, C337S)

CREB bZip triple Cys mutant

Vector:  pMCSG7

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 LB 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, transfer 50 ml culture into 1 L LB plus ampicillin.
5. Shake at 225 rpm, at 37 ºC and monitor cell growth by measuring A600.
6. When A600 reaches 0.8 – 1.0, lower temperature to 20 ºC and shake for 30 min.
7. Add 1 mL of stock IPTG and shake culture at 20 ºC overnight.
8. Harvest cells by centrifuging at 4 ºC for 30 minutes.
9. Store cell pellet at – 80 ºC freezer.

**PURIFICATION**

**Buffer A:**

25 mM Tris, 500 mM NaCl, 1 mM TCEP, 8M Urea, pH 8.0

**Buffer B:**

25 mM Tris, 1 M NaCl, 1 mM TCEP, 8M Urea, pH 8.0

**Elution Buffer:**

25 mM Tris, 500 M NaCl, 1 mM TCEP, 6M Urea, 0.5 M Imidazole, pH 8.0

**Dialysis Buffer:**

25 mM Tris, 200 mM NaCl, 1mM TCEP, pH 8.0

Sterile filter all buffers before use, make fresh each time.

## Lysis procedure

1. Resuspend cell pellet from 1 L culture in 80 mL of buffer A.
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.

**Nickel affinity purification procedure**

(ALWAYS KEEP SAMPLES ON ICE!)

**Prepare nickel resin**

1. Get 8 ml of resin into bio-rad yellow column.
2. Wash resin with 10 CV of water.
3. Incubate resin with 5 CV of 100 mM Nickel sulfate at room temperature for 30 min.
4. Wash resin with 10 CV of water.
5. Equilibrate resin with 5 CV of buffer A.

**Purification procedure**

1. Incubate resin with soluble supernatant at 4 ºC for 30 min.
2. Collected flow through.
3. Wash with 10 CV buffer A and collected wash 1.
4. Wash with 10 CV buffer B and collected wash 2 (to remove DNA contamination).
5. Wash with 2x10 CV buffer A plus 10 mM imidazole and collect wash 3 and 4.
6. Elute with 6 × 10 mL elution buffer and collect E1, E2, E3, E4, E5, and E6.
7. Run gel and combine elutions containing CREB protein.
8. Dialyze against 1 L of dialysis buffer at room temperature overnight.
9. Add 2 ml of TEV and shake at room temperature for at least 6 hrs.

**Regenerate nickel resin**

* 1. Washed resin with 10 CV of water
  2. Incubated resin with 5 CV of 100 mM EDTA at room temp for 30 min
  3. Washed resin with 10 CV of water
  4. Washed resin with 5 CV of 0.5 M NaOH
  5. Washed resin with 10 CV of water
  6. Incubated resin with 5 CV of 100 mM Nickel Sulfate at room temp for 30 min
  7. Washed resin with 10 CV of water
  8. Washed resin with 5 CV of dialysis buffer

**HPLC procedure**

1. Incubate TEV treated protein with nickel resin for 1 hour at 4 ºC (to remove TEV, His-tag, and uncut protein).
2. Collect flow through, filter by syringe filter (0.22 uM) and subject to HPLC purification.
3. Wash resin with 45 ml of dialysis buffer, collected wash filter by syringe filter (0.22 uM) and subjecte to HPLC purification.