Purification of CBP KIX/7

**Vector:**

KIX pMCSG7 in BL21(DE3) (Senthil used CODONPLUS cells but Jenny didn’t and got good expression) competent cells

**Expression:**

* Plasmid Conc: 5 ng/ul.
* Transformed CBP KIX/7 in LB/Amp/ CHL.
* Grew 60ml LB culture at 37°C for about 4 hours.
* Used 60ml of the culture to inoculate in 1L

culture of LB/Amp/ CHL and was grown till

OD600 reached ~1.2. (about 4-5 hrs).

* Induced with 1.2mM IPTG and overexpressed at

37°C for 4 hours.

* Harvested the cells at 6K/30’/4°C.

**Purification:**

**Lysis Buffer**

50mM Tris

8M Urea

0.5M NaCl pH 8

**Wash/Dialysis Buffer**

50mM Tris

0.5M NaCl pH 8

## Cell lysis:

* Resuspended cell pellets from 1L culture in 40 mL of Lysis Buffer.
* Added 800µL of 10mM PMSF, 80µL pepstatin A, 80µL leupeptin. Incubated at 4°C for 10 min.
* Sonicated for 3x5 minutes with 1 second on, 5 seconds off, 30% Amplitude.
* Centrifuged 30 minutes at 12000 RPM.

**Purification:**

1. Prepared column with 12ml of Nickel His-Select resin.
2. Washed with 4x25ml of water.
3. Charged the Resin with 2x25ml of 100mM Nickel Sulfate.
4. Washed with 4x25ml of water.
5. Equilibrated with 25ml of Lysis Buffer.
6. Incubated resin with soluble supernatant for 1hour.
7. Washed with 4x25mL of Equilibration Buffer. Collected Washes 1,2,3 and 4.
8. Eluted with 2x25ml of Elution buffer. Collected Elutions 1 and 2. Ran gel.
9. Dialysed against 1L of Dialysis Buffer for 4 hours at Room temperature.
10. Incubated 6 ml of TEV Protease overnight at Room Temperature. Ran gel.
11. Washed the resin extensively with water and equilibrated with 25ml equilibration buffer.
12. Loaded the TEV cut protein into the resin and incubated for 30 min at 4°C.
13. Collected Flowthrough and Ran gel.
14. Injected the Flowthrough onto the HPLC.

