Purification of WDR5 (2-334)

**Vector:**

pMCSG7 WDR5 in BL21 (DE3) competent cells

**Expression:**

* Transformed pMCSG7 WDR5 in LB/Amp
* Grew 50 ml LB culture at 37°C for about 4 hours.
* Used 50 ml of the culture to inoculate in 1 L culture of TB/Amp
* Induced with 1 mM IPTG when OD600 reached ~1.2 and overexpressed at 16°C overnight.
* Harvested the cells at 6K/30’/4°C.

**Purification:**

**Lysis Buffer**

50mM Tris pH 8

0.2M NaCl

**Wash Buffer – high salt**

50mM Tris pH 8

1M NaCl

**Elution Buffer – high**

50mM Tris pH 8

200mM NaCl

0.5M Imidazole

## Cell lysis:

* Resuspended cell pellets from 1L culture in 80 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 15ml 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 30 min.
7. Washed with 3x25mL of lysis Buffer. Collected Washes 1, 2 and 3.
8. Washed with 2x25mL of High Salt Wash Buffer. Collected Washes 4 and 5.
9. Eluted with 5 x 15ml of Elution buffer. Collected Elutions. Ran gel.
10. Pooled elution 1, 2 and 3. Concentrated down to about 5 ml using centrifuge concentrator.
11. Centrifuged at 12000 rpm for 15 min at 4 °C.
12. Injected 400 ul of supernatant onto the FPLC/sup200, using buffer 20 mM sodium phosphate, pH 7.2, 150 mM NaCl).