His-tag Purification of RF1(Byron Hetrick)

Cell Culture:

- Inoculate 5mL LB broth (100µg/mL kanamycin = 20µL of 25mg/mL kanamycin stock) from frozen cell stock. Incubate on 37^{o}C shaker overnight.
- Inoculate 500mL LB (100µg/mL kanamycin = 2mL of 25mg/mL kanamycin stock) with 1mL saturated overnight culture.
- Grow on 37^{o}C shaker until OD_{600} of 0.6 is reached.
- Induce with 1mM IPTG (120mg IPTG for 500mL culture)
- Incubate culture 4-5 hours on 37^{o}C shaker
- Harvest cells by centrifuging 4000 x g for 20min.
- Freeze cell pellets at -20^{o}C

Preparation of Cleared Lysate:

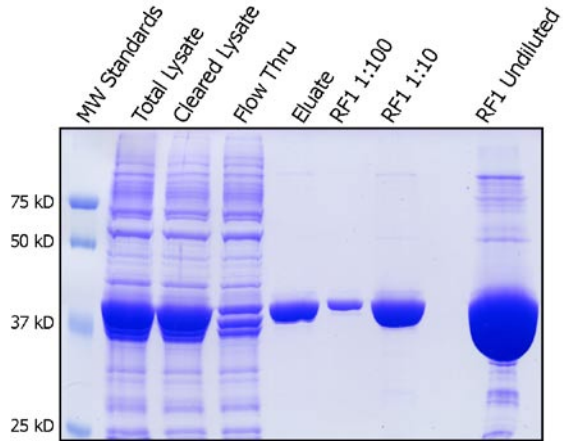
- Thaw frozen cell pellet on ice
- Resuspend in 30mL Lysis Buffer
- Add 30mg lysozyme
- Incubate on ice 30min
- Sonicate on ice:
    - 8 seconds pulse / 8 second rest for 1 min. sonication time
    - Repeat 2X with 1min. rest between each repetition
    - Save 40uL of Total Lysate for gel analysis
- Centrifuge lysate 10,000 x g 30min 4^{o}C
- Remove 40µL for gel analysis (cleared lysate)

Column Purification:

- Pour column with 5mL 50% Ni-NTA slurry (or use regenerated column)
- Wash Ni-NTA column with 5 column volumes (CV) (25mL) of lysis buffer
- Apply cleared lysate to column and wash with 10 CV wash buffer (50mL)
    - Save 40µL of Flow Thru for gel analysis.
- Elute with elution buffer
    - Collect 30 X 1.5mL fractions
- Analyze samples with 10% SDS-PAGE
    - Mix 5µL of Total Lysate, Cleared Lysate, and Flow Through with 1µL 6X Loading Buffer
    - Mix 10µL of each fraction (or every other fraction) with 2µL 6X Loading Buffer

Concentration and Storage:

- Pool fractions containing RF1 and concentrate in Amicon 10kDa cutoff filter to less than 1mL
- Add Storage buffer up to 15mL and concentrate down to less than 1mL
- Repeat wash 2 more times (final dilution of > 3,000-fold is achieved)
- Aliquot RF1 10µL per tube and flash freeze in liquid nitrogen
- Store at -80^{o}C
- Quantitate by Bradford assay.



Buffers and Solutions:

Lysis Buffer:

50mM	$NaH_2PO_4 \bullet H_2O$	6.90 g
300mM	NaCl	17.54 g
10mM	Imidazole	0.68 g

→ Dissolve in 800mL water
→ Adjust pH to 8.0 with NaOH
→ Raise final volume to 1L

Wash Buffer:

50mM	$NaH_2PO_4 \bullet H_2O$	6.90 g
300mM	NaCl	17.54 g
20mM	Imidazole	1.36 g

→ Dissolve in 800mL water
→ Adjust pH to 8.0 with NaOH
→ Raise final volume to 1L

Elution Buffer:

50mM	$NaH_2PO_4 \bullet H_2O$	6.90 g
300mM NaCl		17.54 g
250mM Imidazole		17.0 g

→ Dissolve in 800mL water
→ Adjust pH to 8.0 with NaOH
→ Raise final volume to 1L

Storage Buffer:

		Stock	Volume
50mM	Tris-Cl pH 8.0	1M	5mL
300mM	NaCl	5M	6mL
	Water		89mL
			100mL