6xHis-tagged EBNA-1 NΔ455 Purification Ver.1 using Qiagen Ni-NTA Column under Native Conditions

A. *Buffers*:

1. Lysis Buffer (1 liter)

50 mM NaH2PO4	6.90g NaH2PO4.H2O (MW 137.99g/mol)
300 mM NaCl(up to 2M)	17.54gNaCl(MW 58.44) or 60ml 5M
10 mM Imidazole (up to 100mM)	0.68g (MW 68.08)
10 mM BME (up to 20 mM)	0.69 ml stock (14.4M)

Adust pH to 8.0 using NaOH

2. Washing Buffer (1 liter)

50 mM NaH2PO4	6.90g NaH2PO4.H2O(MW 137.99)
300 mM NaCl(up to 2M)	17.54g NaCl (MW 58.44) or 60ml 5 M
20 mM Imidazole(up to 100mM)	1.36g Imidazole (MW 68.08)
10% Glycerol	100ml 100% stock
10 mM BME	0.69ml stock (14.4M)

Adjust pH to 8.0 by NaOH

3. Elution Buffer (1 liter)

50 mM NaH2PO4	6.90g NaH2PO4.H2O(MW 137.99)
300 mM NaCl	17.54g NaCl (MW 58.44) or 60ml 5 M
250 mM Imidazole	17.00g Imidazole (MW 68.08)

Adjust pH to 8.0 by NaOH

B. Preparing cleared lysates under Native Conditions

1.Thaw the cell pellet for 15' on ice and resuspend the cells in lysis buffer at 2-5 ml per gram weight (I use 10ml for 500ml culture)

2. Add lysozyme to 1mg/ml and 1xprotease inhibitors and incubate on ice for 30'

3.Sonicate on ice (use 6x10" bursts with a 10" cooling period)

4. Centrifuge lysate at 10,000xg for 20' at 4^0C to pellet the cellular debris and save supernatant.

5. Add 5ul 2xSB to 5ul Supernatant and store at -20^0C for SDS-PAGE analysis

C. Batch purification under Native Conditions

1. Add 2.5 ml of the 50% Ni-NTA slurry to 10 ml cleared lysate and mix gently by shaking at 4^0C for 60'

2. Load the lysate-Ni-NTA mixture into a column with the bottom outlet capped (5ml per column)

3. Remove bottom cap and collect the column flow through

4. Wash twice with 4ml washing buffer every time for each column and Save Wash

5. Elute the protein 4 times with 0.5 ml elution buffer per column each time, collect in 4 tubes and analyze by SDS-PAGE (run 5ul, 0.05%)