Purification Protocol for His6-Sds3 CCSID (aa 43-234; pMCSG vector) (20 °C overexpression)

Prepare Following Buffer Solutions (sterile filter):

→ Native Buffer:
- *50 mM Tris
- *200 mM NaCl
- *2 mM TCEP
- *pH 8

→ High Salt Buffer:
- *50 mM Tris
- *1 M NaCl
- *2 mM TCEP
- *pH 8

→ 6M Gdn.HCl Buffer:
- *50 mM Tris
- *200 mM NaCl
- *2 mM TCEP
- *6 M Gdn.HCl
- *pH 8

→ Elution Buffer:
- *150 mM EDTA
- *200 mM NaCl
- *2 mM TCEP
- *6 M Gdn.HCl
- *50 mM Tris
- *pH 8

→ Prepare Ni^{2+} column
- → Wash resin with 3 column volumes of Gdn.HCl buffer
- → Wash resin thoroughly with UV/UF H_2O (10 column volumes)
- → Charge resin by adding 5 column volumes 0.1 M Ni^{2+} solution
- → Wash resin with UV/UF H_2O
- → Wash resin with column buffer (native buffer)
- → Wash resin with UV/UF H_2O
- → Resin is now ready for use

Protocol:

1) Resuspend 500 ml pellet in 30 ml Gdn.HCl buffer (pellet from 1 liter culture = 60 ml buffer)
2) Add protease inhibitors (scale accordingly for larger pellets)
    - *300 μl PMSF
    - *300 μl Triton X-100
    - *30 μl leupeptin
    - *30 μl pepstatin
3) Sonicate 1 hour – 1 second on, 5 seconds off (preset program number 001)
4) Centrifuge 30 minutes @ 12,000 rpm
5) Incubate soluble supernatant with prepared Ni^{2+} column
    - *1 hour @ 4 °C*
6) Collect flow-through
7) Wash with Gdn.HCl buffer (30 ml)^
8) Wash with native buffer (30 ml)^
9) Wash with high salt buffer (30 ml)^
10) Wash with native buffer (30 ml)^
11) Elute with elution buffer
    - *Incubate 10 ml with resin for 10-15 minutes @ 4 °C
    - *Collect elution
    - *Repeat 3 times
12) Combine elution 1 and 2, filter, inject onto HPLC (program: TORC_45C for column 080408)

Notes:

^ = For a 500 ml or 1 l pellet, use 30 ml washes; increase accordingly for larger pellets

* = Dialysis buttons for gel samples
    -- Remove 100 μl of each sample and place in dialysis button
    -- Place in water for minimum 3 hours to remove Gdn.HCl
    -- Run SDS-PAGE gel