

His-F3-Talin Purification ¹⁵N/¹³C Labeled

Purification:

- 1) Cells are pLysS, which means that they produce lysozyme and do not require further lyses methods. Resuspend cells with Binding buffer.
- 2) Add 10mM MgCl₂ (1M stock). Add 40μM DnaseI (1mg/ml stock). Rock for 15min. Save 10μl for running on a gel.
- 3) Spin 16Krpm, 15min, 4°C. Save 10μl supernatant for running on a gel. Resuspend pellet. Save 10μl for running on a gel.
- 4) While spinning the lysate, prepare Ni column by washing with binding buffer.
- 5) Flow the supernatant over the column. Save 10μl flow through for gel.
- 6) Wash 20x column volume with Binding Buffer. Save 50μl wash for gel.
- 7) Elute with Elution Buffer. Do a quick coomassie protein assay to determine the peak. Pool the peak. Take 20μl for gel.
- 8) Dilute immediately with Dialysis Buffer. Dialize with 3 buffer changes one hr between each.
- 9) Run SDS PAGE.

Buffers:

8X Binding Buffer (High Salt)

40mM Imidazole
4M NaCl
160mM Tris-HCl, pH 7.9

8X Wash Buffer (Low Salt) A

480mM Imidazole
1.2M NaCl
160mM Tris-HCl, pH 7.9

Elution Buffer

300mM Imidazole
150mM NaCl
20mM Tris-HCl, pH 7.9