H3 (13-135) fragment purification protocol

Wash Buffer TW buffer unfolding buffer

50 mM TrisHCl 50 mM TrisHCl 50 mM TrisHCl

100 mM NaCl 100 mM NaCl 150 mM NaCl

1mM EDTA 1mM EDTA 6 M GuHCl

1mM benzamidine 1mM benzamidine 10 mM DTT

pH 7.5 1% (v/v) Triton X-100 pH 7.9

pH 7.5

Ni2+ wash buffer cleavage/unfolding buffer Ni2+ elution buffer

50 mM TrisHCl 2 M urea 2 M urea

150 mM NaCl 150 mM L-Arg 150 mM L-Arg

6 M GuHCl 10 mM L-Cys 10 mM L-Cys

5 mM imidazole 50 mM HEPES 50 mM HEPES

pH 7.9 150 mM NaCl 150 mM NaCl

2 mM DTT 2 mM DTT

pH 6.8 250 mM imidazole

pH 6.8

1. Transform 100 ng of expression plasmid (pET28bHisSmt3-H3-13to135) for 6xHis-SUMO-H3(13 to 135) into Rosetta 2 (DE3) pLysS expression cells using standard chemical transformation procedure. Plate on KAN/CAM plates.
2. Prepare 10 mL starter culture (10 mL LB, KAN, CAM) by inoculating media with an individual colony from transformation plate of expression cells and incubate overnight at 37 ˚C, shaking.
3. Inoculate flask containing 1 L LB with KAN, CAM with 10 mL overnight starter culture. Incubate culture while shaking at 37 ˚C until OD600 = 0.6–0.9 (~3 hrs).
4. Induce with a final concentration of 0.4 mM IPTG for 3 hrs at 37 ˚C, shaking.
5. Harvest cells by centrifugation at 4000 RPM at 4 ˚C for 15 min. Remove supernatant and collect pellet into 50 mL conical tube. Flash freeze using liq. N2 and store at -80 ˚C.
6. Thaw cells and add 30 mL wash buffer to resuspend cells, keep on ice.
   1. Note: Purify pellets from 1 L culture separately.
7. Sonicate cell suspension on ice using power output 7 and 50% duty for 6 cycles of 20 sec pulse and 1 min rest.
8. Centrifuge lysed cell suspension at 35000 RPM for 30 min at 4 ˚C.
9. Remove supernatant by pipette to obtain pellet containing inclusion bodies of H3.
10. Wash pellet by crude resuspension with 25 mL TW buffer. Centrifuge at 35000 RPM for 10 min at 4 ˚C, then remove supernatant. Repeat this step once with TW buffer then twice with wash buffer. Pellet can be stored at -20 ˚C.
11. Add 250 µL DMSO to the (thawed) pellet at RT and thoroughly mix and mince using a spatula. Incubate at RT for 30 min.
12. Add a small stir bar to the tube containing the pellet and add 30 mL of unfolding buffer. Let stir at RT for 5-6 hours or overnight to extract H3.
13. Centrifuge extraction solution at 35000 RPM at RT for 30 min to remove undissolved material. Obtain supernatant (extracted H3) by pipette.
14. Purify H3 from supernatant using Ni2+ affinity chromatography at RT. Add supernatant to 4 mL Ni2+-NTA resin pre-equilibrated with unfolding buffer in a conical. Incubate for 1 hr at RT while turning.
15. Apply incubation mixture to gravity column and collect flowthrough.
16. Wash resin with 80 mL Ni2+ wash buffer.
17. Wash resin with 20 mL cleavage/dilution buffer.
18. Batch elute (incubation with resin for 10 min turning) three times with 10 mL Ni2+ elution buffer.
    1. Use nanodrop to estimate H3 concentration in each elution.
    2. Can store elutions at 4 ˚C.
19. Use SDS-PAGE to check samples from the purification process.
20. Cleave 6xHis-SUMO from 6xHis-SUMO using SenP1:
    1. Dilute elutions using cleavage/dilution buffer such that [6xHis-SUMO] < 0.25 mg/mL and [imidazole] < 50 mM.
    2. Add SenP1 to a final concentration of 0.03 mg/mL.
    3. Incubate reaction solutions at RT overnight while turning.
    4. Can store reactions at 4 ˚C.
21. Visualize cleave using SDS-PAGE.
22. Separate H3 from 6xHis-SUMO and His-tagged SenP1 in cleavage reaction using Ni2+ affinity chromatography at RT. Add reaction solutions to 3 mL Ni2+-NTA resin pre-equilibrated with cleavage/dilution buffer in conicals. Incubate for 1 hr at RT while turning.
23. Apply incubation mixture to gravity column and collect flowthrough that contains H3.
24. Elute using 10 mL Ni2+ elution buffer and collect elution that contains SUMO and SenP1.
25. Can store solutions at 4 ˚C. Visualize separation of H3 from SUMO and SenP1 using SDS-PAGE.
26. To load H3 solution onto HPLC: precipitate H3 flowthrough solution using TCA protein precipitation:
    1. Prepare 100% TCA (w/v) and chill on ice.
    2. Add TCA to a final concentration of 15% to protein solution and incubate on ice for 30 min.
    3. Centrifuge sample at 3000 RPM at 4 ˚C for 10 min. Remove supernatant by pipette.
    4. Wash pellet with cold milliQ water to remove TCA.
    5. Centrifuge sample at 3000 RPM at 4 ˚C for 10 min. Remove supernatant by pipette.
    6. Store pellet at 4 ˚C.
27. Purify H3 using RP-HPLC with column ZORBAX SB-C18 and with a 40-60% acetonitrile in 0.1% TFA gradient.
    1. Resuspend pellet in 40% MeCN and 0.1% TFA to load 10-12 mg of protein in 10 mL for one run.
    2. Flow rate: 4.7 mL/min with fraction collection every 50 sec for 83 min.
    3. H3 elutes at approximately 40-50 min.
28. Visualize small samples from fractions from peak using SDS-PAGE.
29. Collect and combine fractions containing pure H3. Blow air over solution to remove MeCN (~50% of solution).
30. Lyophilize H3 solution and determine yield.
31. Resuspend using water and aliquot to needed amounts of H3. Lyophilize aliquots then store at -80 ˚C.