

Small Scale Purification of Secreted His-Tagged Protein From Conditioned Medium

1. Take 15ml of conditioned medium and transfer into 15ml tube.
2. Clear the medium by spinning down 20'/4C/11,000rpm.
3. Carefully transfer the medium to a new 15ml tube.
4. Adjust the medium by adding Tris buffer (pH 8.0) to a final concentration of 20mM, and NaCl₂ to a final concentration of 300mM.
5. Wash 50ul of Nickel-NTA beads (50% slurry) to remove traces of Ethanol: Add 50ul of the Nickel slurry into an Eppendorf tube and add 1ml **wash buffer**. Spin down 2min/3000rpm. Repeat wash at least two more times, and re-suspend the beads in final volume of 50ul of **wash buffer**.
6. Add the beads to the conditioned medium and swirl for 1h/4C
7. Spin down at 3000rpm/4C/5min
8. Transfer medium into a new tube
9. Wash beads with 5ml wash buffer containing 5mM imimidazol and spin again.
10. Repeat steps 8-9 once more, and save the washes in separate tubes.
11. Re-suspend the beads in 100ul wash buffer containing 5mM imimidazol, and transfer into an eppendorf tube.
12. Spin again at 300rpm, and remove 3rd wash into another tube.
13. Elute by adding 100ul of wash buffer containing 250mM imimidazol, **DO NOT** swirl, just agitate gently and incubate for 5 min at Room Temperature.
14. Spin down tube at 3000rpm, and transfer elution#1 into a new tube.
15. Repeat steps 13-14 two more times, to obtain elutions #2 and #3.
16. Load all washes and elution fractions on SDS-page gels for both western and coomassie. (Add 10ul from each 100ul elution, and as much as you can from the unbound medium and the 5ml washes) Also: Do not forget to re-suspend the beads in 100ul of 1x loading buffer and load 10ul on the gel.

Wash buffer: 20mM Tris pH 8.0 ; 300mM NaCl₂;