Protein Purification

This protocol states the steps for the purification of a His-tagged protein from a protein extract.

1. Wash a silica column with distilled water previously.
2. Take 1/1000 volumes of the bacterial culture of HisPur NiNTA-Agarose and place it in a microcentrifuge tube.
3. Add a volume of distilled water to the tube, mix, and centrifuge 1 minute at 800 rpm.
4. Discard the liquid phase, and repeat the water wash three times.
5. Repeat the washes three times, using 10mM imidazol solution.
6. Discard the imidazol, and add the resin to the protein extract.
7. Incubate in a tube rotator for 3h at 4°C.

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8. Pass the extract with the resin through the silica column, collect the liquid and label it as “non bound”. Use it to wash the walls of the tube and pass it again.
9. Add 10x the volume of resin of imidazol 10mM to the silica gel. Collect in a microtube.
10. Repeat the wash with 30mM, 50mM, 100mM, 300mM and 500mM solutions.
11. Run a SDS-PAGE to determine at which concentration of imidazol the background proteins are removed (wash concentration) and at which the protein of interest is collected (elution concentration).
12. Should you need to repeat the purification, you can just use the wash and elution solutions.

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