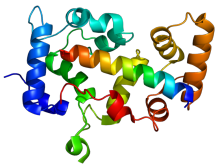
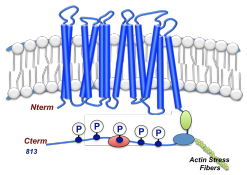


Wash, Strip and Reload NTA Ni Bead Protocol



INTRODUCTION

Ni-NTA nickel is a stable resin easily reused. Most manufacturers recommend using 4-8 times before regeneration. If the resin changes from light blue to brownish-gray the nickel has been lost or changed its oxidations state and will no longer bind His tagged proteins.

Routine Handling

Ni-NTA matrices are stable under a wide variety of conditions and need not be refrigerated, except to inhibit growth of microorganisms for long-term storage. After use they should be washed for 30 minutes with 0.5M NaOH. Ni-NTA matrices should be stored in 30% ethanol to inhibit microbial growth. The matrix can be stored for up to one week in any of the denaturing buffers.

Buffers (Volumes are for every 100 ml of beads):

- 400 ml Regeneration Buffer (6 M Guanadine Chloride, 0.2 M acetic acid in water).
- 300 ml 2% SDS in water
- 100 ml each 25, 50, 75% ethanol in Water.
- 500 ml ethanol
- 500 ml 100 mM EDTA in water (pH 8.0 ~ EDTA will not fully go into solution until pH is adjusted, use mild heat to help dissolve while stirring).
- 100 ml 100 mM $NiSO_4$ in water

Use a wide column to speed things along. Alternatively set up a Buchner funnel but allow each step to "mix" or incubate with the beads for 3-5 min before washing.

Procedure:

1. Wash the column with 2 volumes of Regeneration Buffer.
2. Wash the column with 5 volumes of H_2O.
3. Wash the column with 3 volumes of 2% SDS.
4. Wash the column with 1 volume of 25% EtOH.
5. Wash the column with 1 volume of 50% EtOH.
6. Wash the column with 1 volume of 75% EtOH.
7. Wash the column with 5 volumes of 100% EtOH.
8. Wash the column with 1 volume of 75% EtOH.
9. Wash the column with 1 volume of 50% EtOH.
10. Wash the column with 1 volume of 25% EtOH.
11. Wash the column with 1 volume of H_2O.
12. Wash the column with 5 volumes of 100 mM EDTA, pH 8.0.
13. Wash the column with H_2O.
14. Recharge the column with 2 volumes of 100 mM NiSO4.
15. Wash the column with 2 volumes of H_2O.
16. Wash the column with 2 volumes of Regeneration Buffer.
17. Equilibrate with 2 volumes of a His Binding Buffer (see His Tag Purification)