His tag fusion protein induction/purification protocol

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A: Reagents and solution

8x binding buffer(4M NaCl, 160mM Tris-HCl, 40mM imidazole, pH7.9)

NaCl	58.44g
1M Tris-HCl	40ml
Imidazole	0.68g
DDH_2O	250ml

8X Wash buffer (4M NaCl, 160mM Tris-HCl, pH7.9, 480mM imidazole)

NaCl	58.44g
1.0M Tris-HCl	40ml
Imidazole	2.72g
DDH_2O	250ml

4X elute buffer (4M imidazole, 2M NaCl, 80mM Tris-HCl, pH7.9)

Imidazole	68.08g
NaCl	29.22g
1.0M Tris-HCl	20ml
DDH_2O	250ml

4X Strip Buffer(2M NaCl, 400mM EDTA, 80mM Tris-HCl pH7.9)

NaCl	29.22g
1.0M Tris-HCl	20ml
0.5M	200ml
DDH_2O	250ml

8X Charge Buffer (400mM NiSO4)

NiSO4	26.2g
DDH_2O	250ml

Lysis Buffer (before use: add proteinase inhibitor 1tablet/10 ml and PMSF (100X))

1.0M Tris-HCl pH7.5	5.0ml
5M NaCl	25ml
Glycerol	25ml
Triton-100	250ul
DDH_2O	250ml

B: Protocol:

Transformation of bacterial for you target DNA and grow overnight at 37°C with shaking. **Grown culture: LB = 1:50,** shaking culture at 37°C 2-3 hours, then add IPTG(0.5mM).
Incubate with shaking overnight.

1. Harvest cells by spin at Max speed 30min. Decant supernatant. Allow cells to drain as completely as possible. Resuspend cells in 10ml ice-cold lysis buffer (per 100ml culture volume).

2. Sonicate 4 times, each 10-15sec. (set 5)

3. Spin lysate at 14000rpm for 20min. Collect supernatant to a new tube.

Gently mix bottle of His-Bind Resin until completely suspended. Transfer desired amount to a 15ml tube (0.5-0.8ml/100ml cell culture)

Centrifuge for 1 min. at 1000g. Remove supernatant.
Use the following sequence to charge and equilibrate resin. For each wash step, add appropriate buffer, invert tube several times to mix, and spin for 1 min. at 1000g.

- 2 times with 2 volume sterile deionlized water
- 3 times with 2 volume 1X Charge buffer
- 2 times with 2 volume 1X binding buffer

1. Add resin to cell extracts (supernatant). Mix gently by invert tube several times, Incubate 1 hour with rotating at 4°C

2. Spin I min. at 1000g. Discard supernatant**(sample 1)**
3. Wash resin 3 times with 1X binding buffer
4. Wash resin with 1X wash buffer.
5. Elute bound protein 2 times with 1X elute buffer**(sample 2 and 3)**
6. Alternatively 1X strip buffer may be used to elute protein by strip Ni^{2+} from resin**(sample 4)**

Resin regeneration:

His-resin can be regenerated for reuse, wash resin 3 times with strip buffer. The presence of 100mM EDTA in strip buffer will prevent bacterial growth, Store resin in this solution.
Before next time recharge, use water to completely remove EDTA.

Check gel: non-induced bacteria (70ul), induced bacterial (70ul), sample 1*, 2, 3, 4.
*sample 1 must be calculated equal 70ul bacterial culture.