His-tag purification:

Buffer:

1. Buffer R: 50mM TRIS-HCL pH 7,5
   150mM NaCl
   10mM imidazol
   1 mM Dithiothreitol (DTT)
   100µM PMFS -> add always fresh
   add VE

2. Buffer R+: buffer R supplementet with 20mM imidazol

3. Buffer R++: buffer R supplementet with 200mM imidazol

Protocoll:

1. pack column with 2ml Ni-NTA; equilibrate with buffer R (10ml)
2. resuspend pellet in 10-15ml (25ml for 1L pelett) buffer R
3. sonicate on ice with largest tip for 30sec. Repeat sonication again for 30sec. -> let the sample cool down between the two sonication steps
4. transfer to avanti tubes (ja25-50)
5. spin 30 min. at 20 000g and 4°C -> store pelett and use supernatant
6. take 20µl sample + 40 µl 2xSDS
7. apply supernatant to Ni-NTA column in cold room (4°C) at low flow rate (about 0,5 ml/min)
8. collect flow-through and take 20µl sample + 20µl 2xSDS
9. apply flow-through again on the column
10. optional take sample from any washing steps (20µl + 20µl 2xSDS)
11. wash column with 3x with 10ml buffer R-> drain column and add next volume
12. wash column with 10ml buffer R+ (about 1ml/min)
13. elute protein 5x with 1ml buffer R++. Collect 1ml fractions 5x
14. add samples and eluate and fractions on SDS gel with 20µl
15. detaction with KV-CBB