His tag fusion protein purification

1. Transform PRSET plasmids to BL21 (DE3) (from Promega).
2. Under ex 480, pick up bright colonies.
3. Put in 5ml 100uM amp LB medium, shake 250 rpm 37 °C overnight.
4. take bacteria medium, put in 50ml 100uM amp LB medium, shake 250rpm, 37^{o}C, 2-4hr, monitor the OD 600 reading, 0.2-0.4, dilute into (+400ml) to 450ml 100uM amp LB medium, add IPTG to 0.4 mM (variant IPTG concentrations depend on variant proteins) to induce, RT shake overnight.
5. Spin down 6000 rpm, 10min. Discard the medium.
6. Add 8 ml wash buffer (50mM Tris,HCl pH=7.4, 300mM NaCl, 10mM imidazole)+0.25 protease coctail tablet+100uM PMSF, completely re-suspend, gently rock at RT 10min.
7. Use French Press to break the bacteria.
   1. open the valve of nitrogen cylinder.
   2. with control on "OFF " position, set air pressure to 60.
   3. set valve pressure to 0.
   4. move control to "PUMP" position.
   5. wait to hissing to stop, screw loaded syringe onto inlet/outlet fitting.
   6. move control to "FILL".
   7. as soon as syringe is empty, move control to "PUMP", this is one wash, wash the machine 3 times with water, 3 times with wash buffer.
   8. load syringe with sample, move control to "FILL".
   9. as soon as syringe is empty, move control to "OFF".
   10. increase valve pressure to 80.
   11. move control to "PUMP".
   12. slowly decrease valve pressure until sample fills the syringe slowly, after all the samples come out, decrease pressure to 0.
   13. to repeat, go back to step 7.
   14. wash the machine 3 times with wash buffer, 3 times with water, once with 75% ethanol.
   15. decrease air pressure to 0, and close valve of nitrogen cylinder.
8. Transfer the lysis to 35ml centrifuge tube, Spin 10000rpm for 15 min. filter through 0.4 μm filter (optional). Add Ni-NTA agarose beads 0.5 ml, gentle rock at RT 1hr.
9. Set up flow through column, load the Ni-NTA agarose beads sample
10. Rinse 4 times 10ml wash buffer (50mM Tris,HCl pH=7.4, 300mM NaCl, 10mM imidazole).
11. Elute with elution buffer (50mM Tris,HClph=7.4, 300mM NaCl, 100mM imidazole).
12. Dialysis the protein solution 4 °C, 4hr to overnight.
13. Measure the absorbance following the above protocol.
14. YFP (extinction coefficient) EC=77000 M^{-1} CM^{-1} , CFP EC=32500 M^{-1} CM^{-1}, GFP EC=62000 M^{-1} CM^{-1}. So Concentration=reading/EC.