protocol:purification of PreScission Protease

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The plasmids of PreScission Protease are obtained from high-throughput molecular drug screening center . The protein expressed by the recombinant plasmids has a GST tag and is mainly purified by GST affinity chromatography. The PreScission Protease purification protocol is as follows :

1. Transforming PreSeission Protease plasmids into BL21(ED3) Competent Cells.
2. The selected clone was inoculated into a 5mLLB small test tube, cultured at 37 degrees celsius until the bacterial solution was turbid.
3. Transfer the turbid bacteria solution into 800 mLLB medium, and cultured at 37C° until OD600 reached 0.6.Add 0.5mMIPTG and inducing protein expression for 4h.
4. The bacteria were collected by high speed centrifugation at 5500 rpm for 20 min and suspend the bacteria with 1×PBS.
5. After 4 times of high-pressure bacterias broken, use ultrasonic to continue to break the bacteria for 30 min (ultrasonic power 300w, turn it on for 4s and then turn it off for 6s).
6. Centrifuge at 12000rpm for 30 min and hung the supernatant on GST affinity column for 3-4 times.
7. Wash impurities with 1XPBS until G250 detection does not change blue and then wash with 1×PBS containing 500 mM NaCl (500 mM NaCl, 2.7 mM KCl.10 mM Na_2HPO_4, 1.8 mM KH_2PO_4 (pH=7.3)) until G250 detection does not change blue . Alternately wash high and low salt solution for alternately 2-3 times.
8. Dilute 10×GSH to 2×GSH with 1×PBS ,and use it to elute the target protein until G250 does not change blue.
9. Collect the eluent and concentrate to less than 1mL with 15ml and 30KD concentration tubes.
10. Add Ppase solution(50mM Tris-HCl pH8.0 ,150 mM NaCl, 10 mM EDTA,20%Glycerol ,1 mMDTT)to change the protein solution into Ppase solution .
11. The protein was packaged at 4C° with a concentration of 1 mg/ml, 400μl per tube. Cryopreserve protein by liquid nitrogen freezing method and put it in -80C° for later use.