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Protocol status: Working

Preparation and enzyme activity of recombinant protein

PLOS One

Ning Zhang¹

¹China Agricultural University

Wenxue Wu

GUIDELINES

CTSL Expression-1.jpg

MATERIALS

MATERIALS

⊗ Sodium Phosphate monobasic **Contributed by users**

⊗ Ni-NTA Agarose **Qiagen Catalog #30230**

⊗ 10 mM Tris-HCl **Sigma**

⊗ benzyloxycarbonyl-phenylalanyl-arginine 4-methyl-7-coumarylamide (Z-Phe-Arg-MCA) **Contributed by users**

⊗ E-64 **Sigma Aldrich Catalog #E3132**

⊗ pET-28a-c() vectors **Contributed by users**

SAFETY WARNINGS

! wear gloves and masker when do experiments

BEFORE START INSTRUCTIONS

1. search CTSL mRNA of *Sus scrofa* on NCBI
2. design PCR primer pairs

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21727

- 1 ligate CTSL sequence with pET28a vector.
Step 1 includes a Step case.

step case

untitled case

- 2 Grow small culture overnight.
- 3 Inoculate 1ml overnight culture into 100ml 2xYT media in a 500 ml flask.
- 4 Incubate in a 37°C shaker until OD600 reaches 0.6 (About 3.5 hours), and then add 100 mM IPTG 100ul and incubate for 3 hr.
- 5 Pellet the bacteria at 6, 000 rpm for 5-6min, resuspend in 9ml PBS containing protease inhibitors and transfer to 50 ml conical tubes.
- 6 Sonicate the bacterial suspension on ice, 4 times x 1min with 30s rests (powder setting: cup, 10; tip, 5)
- 7 Transfer the resulting sonicate to a 10-15 ml conical tube and incubate with 1ml 10% Triton X-100 for 30min at 4°C on a rocker

- 8** Transfer the lysates to high-speed tubes (usually 12-14ml falcon tubes from BD or VWR), and centrifuge at 11,000rpm at 4°C to remove unlysed bacteria and cell debris from sonicate. Keep the supernatant, and repeat the step 7 once
- 9** Keep the supernatant in 15ml conical tubes
- 10** Add His beads (200-250ul) and incubate for 1h at RT with constant rocking.
- 11** Pellet the beads at 2,000 rpm for 2 min, 4°C, and aspirate the supernatant.
- 12** Wash the beads 3 times with PBS, 5 min each at RT with rocking
- 13** Resuspend in 300ul PBS
- 14** Store the His beads at 4°C