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Working

Preparation and enzyme activity of recombinant protein

PLOS One

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EXTERNAL LINK

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Zhang N, Gao P, Yin B, Li J, Wu T, Kuang Y, Wu W, Li J (2019) Cathepsin L promotes secretory IgA response by participating in antigen presentation pathways during *Mycoplasma Hyopneumoniae* infection. PLoS ONE 14(4): e0215408. doi: 10.1371/journal.pone.0215408

GUIDELINES

CTSL Expression-1.jpg

MATERIALS

NAME	CATALOG #	VENDOR
Sodium Phosphate monobasic		
Ni-NTA Agarose	30230	Qiagen
10 mM Tris-HCl		Sigma
benzyloxycarbonyl-phenylalanyl-arginine 4-methyl-7-coumarylamide (Z-Phe-Arg-MCA)		
E-64	E3132	Sigma Aldrich
pET-28a-c( ) vectors		

SAFETY WARNINGS

wear gloves and masker when do experiments

BEFORE STARTING

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

1 ligate CTSL sequence with pET28a vector.

step case

no description provided

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