PURIFICATION PROTOCOL

1. Cells were collected by centrifugation, and the pellets were stored at −80°C. 1 gram cell pellets were resuspended and lysed by 10 mL extraction solution (8 M guanidine hydrochloride (GdnHCl), 300 mM NaCl, 50mM, pH 7.2). Lysates were incubated at 4°C for 24 h.
2. The insoluble portions of the lysates were removed by centrifuging at 10,000 g, and the supernatants were incubated with 1 mL Ni-NTA resin for 2 h at room temperature. Beads that bound His-tagged protein were then centrifuged, and washed with 20 mL of potassium phosphate buffer (300 mM NaCl, 50 mM K2HPO4/KH2PO4, pH 7.2) for two times. The washed beads were then added with 3 mL potassium phosphate buffer (300mM NaCl, 50 mM K2HPO4/KH2PO4, pH 7.2).
3. The mixed solutions were then loaded on the columns. GdnHCl was further washed away by adding another 12 mL potassium phosphate buffer (300mM NaCl, 50 mM K2HPO4/KH2PO4, pH 7.2). 20 mL washing buffers (50 mM imidazole and 50 mM potassium phosphate buffer, pH 7.2) were passed through the columns to remove contaminated proteins.
4. Proteins were eluted from the column via 1 mL elution buffer (0.3 M imidazole, 300 mM NaCl, and 50 mM potassium phosphate buffer, pH 7.2) appped in six consecutive elution steps.
5. The proteins are made concentrated using dialysis with PBS buffer of pH6.0. Then they are kept in 4℃ for 72h for renaturation.
6. The harvested cell pellet were rinsed by precooled 10mM phosphate-buffered saline (PBS, pH7.4) and resuspended in 80ml binding buffer (50mM nNa2HPO4, 300mM NaCl and 10mM imidazole, pH 8.0)at a 20-fold concentration.
7. The cells are digested by lysosomes in order to get a soluble protein fraction at a final concentration of 30–50 KU per gram of cell pellets at 30°C for 15min.
8. After that, 40 cycles of sonication at 17 watts is applied for 10s each.
9. The lysates were then centrifuged at 14,000 rpm and 4°C for 30min, and the resulting supernatant was co-incubated with Ni-NTA super resins by shaking at 4°C for 4 h.
10. The mixture was then loaded into dispensable plastic columns for chromatography. Non-specific absorbed proteins were rinsed with washing buffer (pH 8.0) (50mM Na2HPO4, 300mM NaCl and 50 mM imidazole） while the specific recombinant proteins were eluted with

12ml eluting buffer (50 mM, 300 mM NaCl and 250 mM imidazole, pH 8.0).