

## High-level Expression, Purification and Characterization of Recombinant Pea Fructose-1,6-bisphosphatase containing C-Terminal Polyhistidine Tag

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Full-length cDNA encoding pea cytoplasmic fructose-1,6-bisphosphatase (cyFBPase) was cloned from a pea cDNA library. The cloned cDNA was introduced into the *E. coli* expression vector pET-28a with C-terminal HisTag sequence. The recombinant cyFBPase was expressed in *E. coli* BL21(DE3) cells in a soluble form, and purified to homogeneity by Ni<sup>2+</sup>-NTA affinity chromatography. The purified recombinant cyFBPase was confirmed by SDS-PAGE and immunoblot analysis using a polyclonal antibody raised against native pea cyFBPase. The recombinant cyFBPase was active at neutral pH and thermostable as other cyFBPases. The activation energy (E) and Arrhenius frequency factor were 6.93 kcal/mol and 2.21 × 10<sup>3</sup>/s, respectively. The K<sub>M</sub> and V<sub>max</sub> values of the recombinant enzyme were calculated as 12.5 M and 45.5 U/mg, respectively