

## IN VITRO PRODUCTION OF L-CYSTEINE USING THERMOPHILIC ENZYMES

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**Key Words:** Thermophilic enzyme, In vitro metabolic engineering, L-Cystein

L-Cysteine (L-Cys) is a commercially important amino acid and widely used in food, pharmaceutical, and cosmetic industries. Commercial production of L-Cys has long been done by an acid-hydrolysis of human hair and animal feather, leading to the generation of a large quantity of hazardous wastes. Although several biotechnology companies have recently launched a fermentative production of L-Cys using engineered bacteria, these processes suffer from the low product titer mainly due to the cytotoxic effect of L-Cys.

To provide an alternative approach for the commercial production of L-Cys, we aimed at the development of a non-fermentative, in vitro manufacturing system using thermophilic enzymes. In this system, enzymes from (hyper)thermophilic bacteria and archaea were assembled to construct an in vitro synthetic pathway for the one-pot conversion of glucose to L-Cys (Figure 1). By using experimentally optimized concentrations of enzymes, L-Cys could be produced at a rate of 0.9 g/L/h with a molar conversion yield of 25%.

Abbreviations: GK, glucokinase; PGI, glucose-6-phosphate isomerase, PFK, phosphofructokinase; FBA, fructose-bisphosphate aldolase; TIM, triose phosphate isomerase; GAPN, non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase; PGDH, phosphoglycerate dehydrogenase; PSAT, phosphoserine aminotransferase; CysS, cysteine synthase (phosphoserine sulfhydrylase). PPK, polyphosphate kinase; NOX, NADH oxidase. Subscripts: Tt, *Thermus thermophilus*; Tx, *Thermoproteus tenax*; Tk, *Thermococcus kodakarensis*; Ap, *Aeropyrum pernix*; Rm, *Rhodothermus marinus*; Tp, *Thermococcus profundus*.

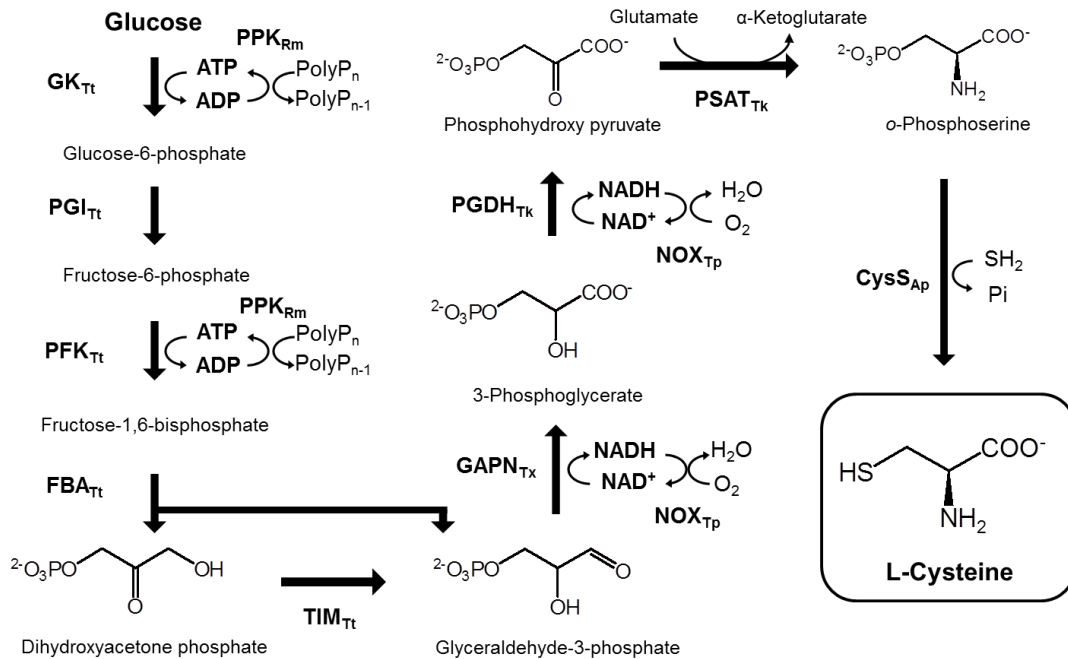


Figure 1 In vitro synthetic pathway for the production of L-cysteine from glucose