# Cloning, Expression And Biochemical Characterization Of Xanthine And Adenine Phosphoribosyltransferases From Thermus Thermophilus Hb8

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## Abstract

Purine phosphoribosyltransferases, purine PRTs, are essential enzymes in the purine salvage pathway of living organisms. They are involved in the formation of C-N glycosidic bonds in purine nucleosides-5′-monophosphate (NMPs) through the transfer of the 5-phosphoribosyl group from 5-phospho-α-D-ribosyl-1-pyrophosphate (PRPP) to purine nucleobases in the presence of $Mg^{2+}$. Herein, we report a simple and thermostable process for the one-pot, one-step synthesis of some purine NMPs using xanthine phosphoribosyltransferase, XPRT or adenine phosphoribosyltransferase, APRT2, from Thermus thermophilus HB8. In this sense, the cloning, expression and purification of TtXPRT and TtAPRT2 is described for the first time. Both genes, xprt and aprt2 were expressed as his-tagged enzymes in E. coli BL21(DE3) and purified by a heat-shock treatment, followed by Ni-affinity chromatography and a final, polishing gel-filtration chromatography. Biochemical characterization revealed TtXPRT as a tetramer and TtAPRT2 as a dimer. In addition, both enzymes displayed a strong temperature dependence (relative activity >75% in a temperature range from 70 to 90 °C), but they also showed very different behaviour under the influence of pH. While TtXPRT is active in a pH range from 5 to 7, TtAPRT2 has a high dependence of alkaline conditions, showing highest activity values in a pH range from 8 to 10. Finally, substrate specificity studies were performed in order to explore their potential as industrial biocatalyst for NMPs synthesis.

## Keywords

Enzymatic Synthesis; Phosphoribosyltransferases; Purine Nucleosides-5′-Monophoshate; Thermostable Biocatalysts.