Enzyme Characterization of Human Inorganic Pyrophosphatase

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Overview

Inorganic pyrophosphatase (IPPase) is an essential, metal-dependent enzyme that is associated with several metabolic pathways including replication and transcription in all domains of life. Cytosolic human IPPase is a Family I IPPase and is suggested to be a biomarker of poor prognosis for gastric cancer. Upregulation of human IPPases is also seen in breast cancer and lung cancer. In the present study, we characterized the human IPPase by enzyme assays. N-terminal his-tagged human IPPase was recombiantly expressed in Escherichia coli Rosetta and purified. Ammonium molybdate colorimetric assay was used to characterize human IPPase at various pH, temperature and metals and its optimal conditions are found to be pH 7.0 at 37 °C in presence of magnesium. Enzyme kinetics are currently underway to determine $K_m$ and $V_m$.

Material and Methods

Enzyme Reaction:
- Buffer
- Metal
- Na PPi
- Human IPPase

Colorimetric Reaction:
- Ammonium Molybdate solution
- Enzyme Reaction mix

37 °C 10 min

Absorbance measured at 660 nm

Results

Effect of pH

Effect of Temperature

Effect of Metal

Enzyme Kinetics

References


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