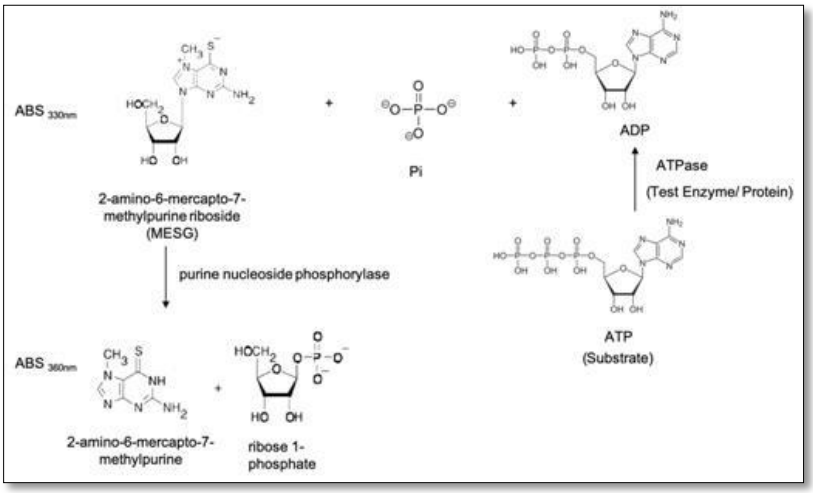
ATPase activity of *Escherichia coli* expressed AAA+-ATPase protein

Scientists at DBT's Regional Centre for Biotechnology (RCB), Faridabad, have provided a detailed protocol to determine the ATPase activity of a recombinant AAA+-ATPase protein (General Control Non-repressible-4 [(GCN4]) by spectrophotometric absorption at 360 nm to measure the accumulated inorganic phosphate. ATPases are the enzymes that breakdown ATP to ADP and release inorganic phosphate (Pi).



In general, the substrate 2-amino-6-mercapto-7-methylpurine riboside (methylthioguanosine, a guanosine analog: MESG) is enzymatically converted in the presence of Pi by purine nucleoside phosphorylase (PNP) to ribose 1-phosphate and 2-amino-6-mercapto-7-methylpurine. The spectrophotometric shift in maximum absorbance at 330nm for the MESG substrate and subsequent conversion product at 360nm due to enzymatic conversion was measured. The GCN4-His-tagged recombinant protein was expressed in *Escherichia coli* BL21 cells and purified using Ni-NTA column.

This purified protein was then used for the quantitation of Pi in solution or the continuous determination of Pi released due to the ATPase activity of GN4, an AAA+-ATPase protein conserved in many eukaryotes, which in plants regulates stomatal aperture during biotic and abiotic stress in plants.

Contact details:

Dr. Deepika Bhaskar; E-mail: deepika.bhaskar@rcb.res.in; Phone No.: 9818497821

Dr. Nidhi Sharma, E-mail: nidhi.sharma@rcb.res.in; Phone number: 8826808920