

### Alternative energy sources for efficient supply of ATP during cell-free protein synthesis

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Maintaining the concentration of Adenosine triphosphate (ATP) is one of the most critical factors for efficient cell-free protein synthesis.

We have examined various chemical compounds carrying high-energy phosphate bonds as alternative energy sources for ATP regeneration protein synthesis system derived from *Escherichia coli*. Specifically, creatine phosphate and four different intermediates of glycolytic pathway (G6P, FBP, 3PGA, PEP) were compared for their capacity to regenerate ATP during cell-free protein synthesis. All of the chemicals examined exhibited different efficiency of ATP regeneration, and accordingly, final quantities of the expressed protein were different, too.

CP was the most effective energy source providing a stable supply of ATP. Among the glycolytic intermediates, 3PGA gave the highest productivity especially in the presence of several cofactors. Approximately 700ug/ml of chloramphenical acetyltransferase (CAT) was produced under an optimized condition utilizing 3PGA. Use of glycolytic intermediates as an energy source also offers a benefit of mimicking in vivo conditions for protein expression.