

## Dual energy-regenerating cell-free protein synthesis system

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The accumulation of inorganic phosphate inhibits protein synthesis in cell-free protein synthesis reactions that are energized by high-energy-phosphate-containing compounds. This study developed a new scheme for supplying energy using a dual energy source to enhance the regeneration of ATP and lower the level of phosphate accumulation. In the proposed scheme, where creatine phosphate and glucose were used as the energy sources, the phosphate released from the creatine phosphate was subsequently used in the glycolytic pathway for the utilization of the glucose, which enhanced the ATP supply and reduced the rate of inorganic phosphate accumulation. When tested against different proteins, the developed method produced 1.5-2.1 times more protein than the previous ATP regeneration methods using a single energy source.