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Expression of reteplase in Escherichia coli BL21 (DE3) using T7 promoter

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Background and Aims: Reteplase is a segment of tissue Plasminogen Activator (t-PA) used for the removal of thrombi in blood vessels. In the present study the cloned reteplase gene was used for its expression in Ecoli strain BL21 (DE3) cells.
Methods: The recombinant plasmid, pET 15b/Reteplase, was transformed into competent E.Coli strain BL21 (DE3) cells. Overnight culture of transformed bacteria was induced by addition of IPTG to the final concentrations of 0.25, 0.5, 1 and 1.5 mM. Also, the effects of temperature, shaking speeds and glucose concentration on the expression of reteplase were examined (25, 30, 37 and 39 °C; 100, 170 and 190 rpm and 0.25, 0.5, 0.75 and 1 mM, respectively). Samples were analyzed by SDS-PAGE and the expression of reteplase was examined.
Results: After obtaining recombinant E. coli cells, the presence of reteplase in these cells was examined by western blotting which revealed that the target protein was expressed as a unique band at 39kD. The purpose of this investigation was to find conditions which produce high levels of reteplase. Maximum amount of protein expression was obtained by the addition of 1mM IPTG, at 37°C, 100rpm of shaking speed and the absence of glucose in the media.
Conclusion: In this study, expression of reteplase in E.coil BL21 (DE3) was optimized.

Keywords: Reteplae, expression, temperature, shaking speed, glucose