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A human full length preparation of ERCC1-XPF suitable to test compounds targeted to DNA repair mechanisms

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Different human recombinant and native preparations of ERCC1-XPF have been obtained in order to assess kinetic parameters of the enzyme and the effect of compounds directed to target DNA repair mechanisms. Truncated recombinant forms of ERCC1-XPF can be obtained in high yield, however, some of these shorter sources of ERCC1-XPF have been found to contain low molecular weight contaminants which are needed for the nuclease activity. A similar scenario has been found for reconstituted native ERCC1-XPF systems where additional enzymes were required for DNA nicking. Here, we decided to express, purify and validate a full length, wild-type recombinant ERCC1-XPF protein from a bicistronic expression plasmid introduced in E. coli (Bowles B. et al Nuc. Acids Res. (2012) 40 (13): e101). The purpose was to obtain a source that could be used to test compounds directed against ERCC1-XPF. Methods: ERCC1-XPF purification involved three main steps: nickel agarose, heparin Sepharose and filtration on a Superdex column. ERCC1-XPF incision activity was measured with a 5'-6-FAM, 3'-dabcyl stem-loop oligonucleotide substrate. Results: a close-to-homogeneity preparation of ERCC1-XPF could be obtained following these procedures with a yield of 20-50 ug protein/L culture. The enzyme showed an specific activity of 2.76 nmol product/min x mg protein, which, although it is somehow lower than a previously reported activity of a similar preparation of the enzyme (8.2 nmol product/min x mg protein), it is still several orders of magnitude higher than the activity found in truncated forms of ERCC1-XPF. Enzyme activity could be inhibited with low concentrations of specific nuclease inhibitors. Conclusion: An active, full length ERCC1-XPF was obtained. Yield and ease of obtaining have to be considered before generalizing its use for high throughput screening.

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