

# Activity of the E75E76 mutant of the $\alpha$ subunit of casein kinase II from *Xenopus laevis*

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The cDNA gene coding for the  $\alpha$  subunit of *Xenopus laevis* casein kinase II was mutated using the overlap extension PCR method. The mutation substituted glutamic acids for Lys75 and Lys76, changing the charge distribution of a very basic sequence found in the  $\alpha$  subunit. Expression of the mutated cDNA in a pT7-7 vector in *E. coli* yielded an active mutant recombinant protein that was extensively purified. This mutant was not significantly affected in its app. Km for casein or a model peptide substrate, nor in its interaction with the activating  $\beta$  subunit. Inhibition by quercetin and by 5,6-dichloro-1- $\beta$ -D-ribofuranosyl benzimidazole was also the same for mutant and wild type subunits. However, the CKII  $\alpha$ E75E76 mutant was at least one order of magnitude less sensitive to inhibition by polyanionic inhibitors such as heparin, poly U, copolyglutamic acid:tyrosine (4:1) and 2,3 diphosphoglycerate. © 1994.