

RECOMBINANT HUMAN GLADULAR KALLIKREIN, A NEW PURIFICATION PROTOCOL.

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The human glandular kallikrein (hK2), an enzyme that belongs to the serine proteinases is highly expressed in the prostate. hK2 is able to liberate prostate specific antigen (PSA) which is used as a marker for prostatic cancer, even with limitations for a precise diagnostic. hK2 has been studied as a putative marker of prostatic pathologies providing, with PSA, a better tool for diagnosis of this disease. An expression system, able to produce milligrams of hK2 per liter of medium was established using the yeast *Pichia pastoris*. Enzymatic activity was determined by fluorometric assay using as substrate ZFR-MCA. For detection of protein, electrophoresis in 12.5% polyacrylamide gel (PAGE) stained by Coomassie blue or silver nitrate and Western blot were used. For purification, gel filtration chromatography was run using Superdex HR-200 column (Amersham). The active fractions were pooled and loaded on a benzamidine-sepharose column, which was eluted by two different protocols. In both protocols, after loading, the column was eluted with 50 mM Tris-HCl pH 8.2 containing 150 mM NaCl (buffer A) and in a second step with 3 mM HCl (buffer B) (protocol 1) or with Afilit (Proteobras) a buffer specific to elute proteins from affinity chromatography (protocol 2). Our results show that the enzyme is homogeneous in PAGE by both protocols, however, the specific activity of the enzyme purified by protocol 2 is approximately 12-fold higher than that from protocol 1.

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