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Expression and fast preparation of biologically active recombinant human coagulation factor VII in CHO-K1 cells

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ABSTRACT. Human coagulation factor VII (FVII) plays an important role in the blood coagulation process and exists in micro amounts in human plasma; therefore, any attempt at the large-scale production of FVII in significant quantities is challenging. The purpose of this study was to express and obtain biologically active recombinant FVII (rFVII) from Chinese hamster ovary K1 (CHO-K1) cells. The full-length FVII cDNA was isolated from a HepG2 cell line and then subcloned in pcDNA3.1 to construct an expression vector, pcDNA-FVII. CHO-K1 cells were transfected with 1 µg pcDNA-FVII. The cell line that stably expressed secretory FVII was screened using 900 µg/mL G418. The FVII copy number in CHO-K1 cells was detected by quantitative polymerase chain reaction (qPCR). The rFVII was purified in ligand affinity chromatography medium. The purified protein was detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot analysis. The biological activity of the purified FVII protein was determined by a prothrombin time assay. Three cell lines that permanently expressed rFVII were screened. The

qPCR results demonstrated that each CHO-K1 cell harbored two FVII DNA copies. The SDS-PAGE and Western blot analysis showed that the purified protein was about 50 kDa. The purity of the target protein was 95%. The prothrombin time assay indicated that the FVII-specific activity of rFVII was 2573 ± 75 IU/mg. This method enabled the fast preparation of high-purity rFVII from CHO-K1 cells, and the purified protein had good biological activity.

Key words: Human coagulation factor VII; Specific activity; Quantitative polymerase chain reaction; Ligand affinity chromatography

