

论著

人组织因子在毕赤酵母中的表达和纯化

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摘要

目的 制备具有凝血活性的重组人组织因子(recombinant tissue factor, r-TF) 用于构建 PT 试剂盒。方法 将人组织因子胞外区编码基因与酵母表达载体重组, 构建酵母表达质粒 pPIC9K-TF, 利用电穿孔法转化宿主菌 *Pichia pastoris* GS115, 转化子经 G418 抗性筛选后, 获得高表达克隆。工程菌在摇瓶中经甲醇诱导, 表达 r-TF, 表达产物经纯化后, 鉴定生物活性。结果 SDS-PAGE 证实表达产物的分子量为 37000-45000, Western-Blot 证明其为人组织因子衍生物, 纯化后, r-TF 经脂化后, 具有促凝血活性, 为研制凝血酶时间测定试剂盒及研究 TF 的构效关系创造条件。结论 用毕赤酵母高效表达具有活性的 rTF, 表达量达到 182mg/L。纯化工艺简便易行, 蛋白回收率高, 纯化后的 rTF 可用于组装 PT 测定试剂盒。

关键词 [组织因子](#); [外源性凝血](#); [毕赤酵母](#); [凝血酶原时间](#)

分类号

Expression and Purification of the Recombinant Human Tissue Factor in *Pichia Pastoris*

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Abstract

Objective To obtain recombinant human extracellular tissue factor (r-TF) which can be used to set up PT kit. Methods Expression plasmid, pPIC9K-TF, was constructed by inserting the sequence encoding human extracellular tissue factor into yeast expression vector pPIC9K and transformed into *Pichia Pastoris* GS115 with electroporation. Having been selected by G418 transformants containing TF cDNA were induced by methanol for the expression of rTF, purified and analysis activity. Results SDS-PAGE showed that the molecular weight of the expression product was about 37000-45000 Dalton. western-blotting indicated that it was human extracellular tissue factor. After phospholipids treatment, purified rTF was able to initiate blood coagulation. Conclusions rTF gene is expressed in *Pichia Pastoris* and the active products are secreted into the medium with concentration up to 182mg/L. The recombinant protein is purified with a simple process and high rate of protein recovery, the purified protein can be used in PT test.

Key words [tissue factor](#) [blood coagulation](#) [Pichia pastoris](#) [Prothrombin time](#)

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