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论文

柱上切除GST标签制备幽门螺杆菌Lpp20蛋白

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摘要:

目的 表达幽门螺杆菌Lpp20-GST融合蛋白,获取切除GST标签的重组蛋白。方法 采用异丙基硫代半乳糖苷(IPTG)诱导重组表达质粒Lpp20/pGEX-4T-1在大肠埃希菌BL21(DE3)中表达,收集菌体并采用反复冻融、溶菌酶裂解及超声破菌3种细胞破碎方法,表达产物在谷胱甘肽琼脂糖树脂4B柱上纯化,利用凝血酶切除GST标签,用鼠抗Lpp20单克隆抗体进行纯化产物western blot鉴定。结果 高效表达出Lpp20-GST融合蛋白,相对分子质量约为4.5 kDa,产物以部分可溶性形式表达,凝血酶成功切除GST标签,纯化产物能被鼠抗Lpp20单克隆抗体识别。结论 凝血酶柱上切除GST标签获得目的蛋白。

关键词: 幽门螺杆菌 Lpp20 凝血酶 纯化 GST标签

Preparation of Lpp20-GST fusion protein of *Helicobacter pylori* with thrombin-cleavage of GST tag on column

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Abstract:

Objective To express Lpp20-GST fusion protein with glutathione-S-transferase(GST)fusion gene expression system and the cleavage of GST-tag on glutathione sepharose 4B column using thrombin. Methods The recombinant expression plasmid Lpp20/pGEX4T-1 was induced in *E.coli* BL21(DE3)by isoproythio β D-galacoside (IPTG)and the bacterial sediment was lysed by repeating freezing and thawing,lysozyme lysis,and ultrasonic wave.The soluble supernatant was loaded on glutathione sepharose 4B column and GST-tag was cleavaged on column using thrombin.Purified Lpp20 was proved by mouse anti-Lpp20 monoclonal antibody(mAb)with western blot. Results The fusion protein Lpp20-GST was partly expressed in soluble form with relative molecular mass of 45 kDa.Thrombin cleavaged GST-tag on column and purified Lpp20 was recognized by mouse anti-Lpp20 mAb. Conclusion Target protein can be obtained by thrombin-cleavage of GST-tag on column.

Keywords: *Helicobacter pylori* Lpp20 thrombin GST tag purification

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