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# 两步柱色谱法分离纯化重组猪β2-肾上腺素能受体

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# Two-step chromatographic method for the separation and pur porcine β2-adrenoceptors

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摘要 β2-肾上腺素能受体是细胞表面受体的一种,它能通过偶联异源三聚体G蛋白将信号转导引入到细胞内部。本实验在成功克隆、表 腺素能受体的基础上,建立了一种两步柱色谱分离纯化目的蛋白质的方法。首先利用Ni2+螯合的高分辨纯化的预带电荷介质Sephar Performance与含有六聚组氨酸标签的蛋白质特异结合的性质,对目的蛋白质进行初步分离,接着运用快流速Q琼脂糖凝胶(Quaterr Sepharose Fast Flow)对其进行进一步的分离纯化。采用该方法得到的β2-肾上腺素能受体蛋白质经十二烷基硫酸钠-聚丙烯酰胺 (SDS-PAGE)和高效凝胶排阻色谱检测其纯度约为95%。结果表明该方法可以对重组猪β2-肾上腺素能受体进行有效的分离纯化。

关键词： 柱色谱 分离 纯化 β2-肾上腺素能受体

Abstract： β2-Adrenoceptors are the members of cell surface receptors which perform their signal transductic interior of the cells by coupling to heterotrimeric G proteins. On the foundation of successful clone and expres adrenoceptors, a two-step chromatographic method using Ni-chelated Sepharose High Performance affinity m Quaternary Sepharose Fast Flow anion exchangers was established to prepare recombinant β2-adrenoceptor expressed in E. coli BL21(DE3) as histidine-tagged protein. In the affinity chromatographic column, the buffer consisted of 20 mmol/L phosphate buffered saline (PBS) containing 500 mmol/L NaCl (pH 7.4), and the buffer consisted of buffer A with the addition of 0.5 mol/L imidazole (pH 7.4); in anion chromatographic column, the b was 20 mmol/L PBS (pH 7.4), and the buffer B was consisted of buffer A with 800 mmol/L NaCl (pH 7.4). The a results of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and high performance size-e chromatography (Shim-pack Diol-300) showed that the purity of obtained β2-adrenoceptors was about 95%. Furthermore, the bioactivity of β2-adrenoceptors was studied by receptor ligand combination test, and the res assured the object protein possessed good bioactivity. Finally the conclusion can be reached that the method effectively separate active recombined β2-adrenoceptors.

Keywords： column chromatography separation purification β2-adrenoceptors

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