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论文

黄曲霉毒素B_1抗原模拟表位高效表达

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摘要:

目的 构建黄曲霉毒素B_1(AFB_1)模拟表位pⅧ噬菌体展示载体,为建立无毒害免疫学检测方法提供依据。方法 构建呈现AFB_1抗原模拟表位的噬菌体载体,并引入肠激酶酶切位点,诱导表达,肠激酶处理重组噬菌体颗粒,酶联免疫吸附法鉴定反应原性。结果 重组质粒酶切谱、PCR扩增结果及测序结果均与设计一致,在pC89载体中插入了GACGACGACGACAAGCATCCTAGTGATCCGCGTCATGGG序列,肠激酶处理后的重组噬菌体颗粒可与AFB_1抗体特异性结合,吸光度(*A*)值可达2.112。结论 成功构建了包含肠激酶酶切位点的AFB_1抗原模拟表位pⅧ噬菌体表达载体,重组噬菌体颗粒经初步表达有一定反应原性。

关键词: 黄曲霉毒素B_1(AFB_1) 模拟表位 pC89 肠激酶

High-density expression of a phagemid for AFB_1 mimotope

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Abstract:

Objective To construct a phagemid for aflatoxin B_1(AFB_1) mimotope with the characteristics of high-density expression and a completely exposed N terminal,and to provide a method for preparing an alternative to AFB_1 artificial antigen.Methods We constructed a phasemid expressing AFB_1 mimotope and enterokinase site;the expression of the phasemid was induced by isopropyl-β-d-thiogalactopyranoside(IPTG).After protein cleavaged by enterokinase,an antibody of AFB_1 was used in enzyme-linked immunosorbent assay(ELISA) to detect the reactionogenicity of the mimotope.Results The results of plasmid restriction endonuclease cleavage,PCR amplification and sequencing were in good accordance with the synthetic sequence;the target sequence GACGACGACGACAAGCATCCTAGTGATCCGCGTCATGGG was inserted and the mimotope cleavaged by enterokinase could be recognized by the antibody of AFB_1,with an absorbance value of up to 2.112.Conclusion A phasemid expressing AFB_1 mimotope exposed to the amino terminal of pⅧ after enterokinase cleavage was successfully constructed.

Keywords: AFB_1 mimotope pC89 enterokinase

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