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α-N-乙酰半乳糖胺酶的表达及活性检测

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Overexpression and characterization of a bacterial α-N-acetylgalactosaminidase

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摘要 为了建立新型α-N-乙酰半乳糖胺酶的筛选、检测方法，实验中提取脑膜金黄杆菌的基因组DNA，以此为模板PCR扩增出α-N-乙酰半乳糖胺酶(A4).将A4克隆至pET-24a载体，转化Bl21表达菌株进行蛋白表达.使用亲和层析方法纯化His-A4酶，选择显色底物验证酶活性.同时，改进了传统的ELISA方法，直接将红细胞膜包被于ELISA检测平板中，以红细胞膜表面抗原作为直接底物，用ELISA方法检测酶活性.此研究建立了新型ELISA实验方法，以此方法验证了A4酶的活性，证明了此酶能够有效降低红细胞表面抗原抗体反应，且具有浓度和时间依赖性.

关键词： &alpha -N-乙酰半乳糖胺酶 重组蛋白 蛋白纯化 ELISA方法

Abstract: The coding sequence of alpha-N-acetylgalactosaminidase (A4) was amplified from genomic DNA of Chryseobacterium meningosepticum and subcloned into pET24a, which was then transformed into BL21(DE3) for overexpression of His-A4. The overexpressed His-A4 enzyme was purified by using affinity chromatography and its activity was comparable to that previously reported by using a conventional method with an artificial substrate. To better measure the activity of α-N-acetylgalactosaminidase in real application, we established a novel method in which we directly used the surface antigen of red blood cell as substrate and applied ELISA to the detection of un-cleaved antigen. The activity of His-A4 was evaluated in the new ELISA method and was demonstrated to be able to decrease the blood cell surface antigen-antibody reaction in concentration- and time-dependent manner.

Key words: α-N-acetylgalactosaminidase recombination protein protein purification ELISA method

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