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建立利用萤光素酶快速检测细胞活性的方法

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Development of a luciferase-based method for rapid cell viability detection

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摘要 运用PCR的方法，从萤火虫萤光素酶基因载体 pGL4.26 扩增萤火虫萤光素酶基因片段，将其插入连接于原核表达载体pET24a 中，构建重组表达载体pET24a-Luc.经酶切鉴定及序列分析后，将重组载体转化到表达菌株大肠杆菌BL21（DE3） 中，获得阳性重组菌BL21/pET24a-Luc.IPTG诱导蛋白高效表达并通过镍柱亲和层析纯化萤火虫萤光素酶.该目的蛋白活性用Bright-Glo TM 试剂进行验证并用于建立一种基于测量ATP含量的检测细胞生物活性的方法.与传统的细胞生物活性检测试剂盒MTT，CCK-8以及Alamar Blue比较，该方法具有反应迅速、活力高、灵敏度好、生产方便的优点，具有实际应用的潜力.

关键词： 萤火虫萤光素酶 蛋白表达 蛋白纯化 细胞活性

Abstract： The firefly luciferase gene was PCR-amplified from plasmid pGL4.26 and subcloned into a bacterial overexpression vector pET24a. Expressed luciferase fusion protein was purified using Ni-NTA affinity chromatograph and its activity was confirmed using Bright-Glo TM kit. Based on the ATP-dependency of luciferase reaction, we developed a cell viability assay, which is faster, more convenient, and more sensitive in detect cell viability than generally used MTT, CCK-8 and Alamar Blue methods.

Key words： firefly luciferase protein expression protein purification cell viability assay

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