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刚地弓形虫苹果酸脱氢酶基因的克隆 表达及免疫原性分析

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Cloning，Expression and Immunogenicity Analysis of Malate Dehydrogenase Gene of Toxoplasma gondii

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摘要 参考文献 相关文章

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摘要 目的 克隆、表达刚地弓形虫苹果酸脱氢酶（TgMDH）基因，并分析其免疫原性。 方法 提取弓形虫RH株速殖子总RNA，根据TgMDH的基因编码序列（GenBank登录号为AY650028）设计引物，RT-PCR扩增产物双酶切后连接入pET30a（+）载体，重组质粒转化入大肠埃希菌（E. coli）DH5α，经双酶切、PCR和测序鉴定阳性菌落。重组质粒pET30a（+）?-TgMDH转化至E. coli BL21，经异丙基-β-D-硫代半乳糖苷（IPTG）诱导表达，十二烷基硫酸钠-聚丙烯酰胺凝胶电泳（SDS-PAGE）结合考马斯亮蓝染色检测表达产物。优化表达条件，获得大量可溶性蛋白。经镍亲和层析法纯化后滴鼻免疫BALB/c小鼠，制备小鼠抗重组rTgMDH蛋白血清。分别以小鼠抗rTgMDH血清和兔抗弓形虫血清为一抗，蛋白质印迹（Western blotting）分析rTgMDH蛋白的免疫原性。 结果 TgMDH基因RT-PCR扩增产物约为951 bp。经双酶切、PCR和测序结果显示重组质粒pET30a-TgMDH构建成功。SDS?鄄PAGE结果显示，经IPTG诱导获得相对分子质量（Mr）约36 000的可溶性重组蛋白。Western blotting分析结果显示，rTgMDH蛋白能被该蛋白免疫的小鼠血清和兔抗弓形虫血清识别。 结论 本研究克隆的TgMDH基因序列能在原核表达系统中高效表达，且具有免疫原性。

关键词： 刚地弓形虫 苹果酸脱氢酶 原核表达 免疫原性

Abstract： Objective To clone and express the malate dehydrogenase（MDH） gene of Toxoplasma gondii， and analyze the immunogenicity. Methods Total RNA was extracted from tachyzoites of RH strain of T. gondii（GenBank accession No. AY650028）. The coding region of TgMDH was amplified with a pair of specific primers. The product of RT?鄄PCR was digested with double restriction enzyme and ligated into pET30a（+） vector. The recombinant pET30a（+）-TgMDH plasmid was transformed into E. coli DH5α. The positive clones were confirmed by the double restriction enzyme digestion， PCR and sequencing. The correct plasmid was transformed into E. coli BL21 and induced by IPTG. The expressed proteins were analyzed by SDS-PAGE. Conditions for expression were optimized. Abundant soluble rTgMDH protein was purified with Ni-NTA affinity chromatography. Mice was intranasally immunized with purified rTgMDH and murine anti-rTgMDH serum was prepared. Western blotting with murine anti-rTgMDH serum and rabbit anti-T. gondii serum was used to analyze its immunogenicity. Results The product of RT-PCR was with 951 bp. The recombinant plasmid pET30a（+）?-TgMDH was confirmed by the double restriction enzyme digestion， PCR and sequencing. A soluble recombinant protein with relative molecular weight of 36 000 was analyzed by SDS-PAGE， followed by coomassie blue staining. Western blotting revealed that rTgMDH can be recognized by murine anti-rTgMDH serum and rabbit anti-T. gondii serum. Conclusion TgMDH gene has been expressed in prokaryotic expression system and shows immunogenicity.

Keywords： Toxoplasma gondii Malate dehydrogenase Prokaryotic expression Immunogenicity

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