中国寄生虫学与寄生虫病杂志
CHINESE JOURNAL OF PARASITOLOGY AND PARASITIC DISEASES
ISSN 1000-7423
CN 31-1248/R
主管：中华人民共和国卫生部
主办：中华预防医学会
中国疾病预防控制中心寄生虫病预防控制所

中国寄生虫学与寄生虫病杂志 » 2012, Vol. 30 » Issue (2) :86-89 DOI:

刚地弓形虫磷酸甘油酸变位酶2基因片段克隆、表达及抗原性分析

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Cloning，Expression and Antigenicity Analysis of Phosphoglycerate Mutase 2 Gene of *Toxoplasma gondii*

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摘要 目的 克隆、表达刚地弓形虫（*Toxoplasma gondii*）磷酸甘油酸变位酶2（*Tg*PGAM2）基因片段，并分析其抗原性。 方法 提取弓形虫RH株速殖子总RNA，逆转录合成cDNA。PCR扩增TgPGAM2基因。扩增产物经双酶切后连接入pET30a(+)载体，重组质粒转化大肠埃希菌（*E. coli*）DH5α，阳性菌落经PCR和双酶切鉴定，并测序。将测序正确的重组质粒pET30a(+)-*Tg*PGAM2转化至*E. coli* BL21并加入异丙基-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。以兔抗弓形虫血清为一抗，蛋白质印迹（Western blotting）分析重组蛋白的抗原性。 结果 PCR扩增产物约为750 bp。菌落PCR、双酶切和测序结果显示，重组质粒pET30a(+)-*Tg*PGAM2构建成功。SDS-PAGE结果显示，经IPTG诱导获得相对分子质量（M_r）约30 000的可溶性重组蛋白。Western blotting分析证实其能被兔抗弓形虫血清识别。 结论 刚地弓形虫RH株*Tg*PGAM2基因片段可在原核表达系统中表达，且该可溶性重组蛋白具有抗原性。

关键词： 刚地弓形虫 磷酸甘油酸变位酶2 基因克隆 原核表达 抗原性

Abstract： Objective To clone and express the phosphoglycerate mutase 2 (PGAM2) gene of *Toxoplasma gondii*, and analyze the antigenicity of the recombinant protein. Methods Total RNA was extracted from *T. gondii* tachyzoites of RH strain and reversely transcribed into cDNA. *Tg*PGAM2 gene was amplified by PCR and cloned into pET30a(+) vector. The constructed pET30a(+)-*Tg*PGAM2 was transformed into *E. coli* DH5α first and selected through the colony-PCR and confirmed by the double restriction enzyme digestion and sequencing. The correct plasmid was transformed into *E. coli* BL21 for expression induced by IPTG and the recombinant protein was further analyzed through SDS-PAGE followed by Coomassie brilliant blue staining. Western blotting assay with rabbit anti-*T. gondii* serum was used to analyze its antigenicity. Results The length of PCR product was about 750 bp and the recombinant plasmid pET30a(+)-*Tg*PGAM2 was successfully constructed. The results of SDS-PAGE and Western blotting revealed that the relative molecular weight (M_r) of the soluble recombinant protein was approximately 30 000 and could be recognized by rabbit anti-*T. gondii* serum. Conclusion The soluble *Tg*PGAM2 protein has been expressed in the prokaryotic expression system and maintains its antigenicity.

Keywords： *Toxoplasma gondii* Phosphoglycerate mutase 2 Gene cloning Prokaryotic expression Antigenicity

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殷丽天1, 王芬2, 孟晓丽2, 王海龙2, 刘红丽2, 申金雁2, 殷国荣2 *.刚地弓形虫磷酸甘油酸变位酶2基因片段克隆、表达及抗原性分析[J] 中国寄生虫学与寄生虫病杂志, 2012,V30(2):86-89

YAN Li-Tian1, WANG Fen2, MENG Xiao-Li2, WANG Hai-Long2, LIU Gong-Li2, SHEN Jin-Yan2, YAN Guo-Rong2 *.Cloning，Expression and Antigenicity Analysis of Phosphoglycerate Mutase 2 Gene of *Toxoplasma gondii*[J] , 2012,V30(2):86-89