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 » 2011, Vol. 29 » Issue (5) :363-367 DOI:

论著

GST沉降技术验证弓形虫醛缩酶与肌动蛋白的相互作用

1 新乡医学院寄生虫学教研室，新乡 453000；2 中山大学中山医学院寄生虫学教研室，广州 510080；
3 新乡医学院药学院，新乡 453000

Protein Interaction between Aldolase and Actin of *Toxoplasma gondii* by GST Pull-down

1 Department of Parasitology，Xinxiang Medical University，Xinxiang 453000，China; 2 Department of Parasitology，Zhongshan School of Medicine，Sun Yat-sen University，Guangzhou 510089，China; 3 College of Pharmacy，Xinxiang Medical University，Xinxiang 453000，China

摘要 | 参考文献 | 相关文章

Download: PDF (266KB) HTML 1KB Export: BibTeX or EndNote (RIS) Supporting Info

摘要 目的 通过GST沉降技术（GST pull-down）验证刚地弓形虫醛缩酶（aldolase）与肌动蛋白（actin）的相互作用。 方法 PCR扩增弓形虫cDNA中aldolase 和actin基因，分别亚克隆至原核表达质粒pGEX?鄄4T?鄄1和pET30a，转化至大肠埃希菌BL21（DE3），1 mmol/L异丙基?鄄β?鄄D?鄄硫代半乳糖苷（IPTG）诱导表达，亲和层析法纯化表达产物。采用腹部皮内多点注射免疫SD大鼠15只，首次免疫Actin-His6蛋白量为200 μg/只，第2次起免疫蛋白量为100 μg/只，共免疫4次，每次间隔7 d，末次免疫后5 d收集心脏血，制备Actin-His6抗血清。以纯化的GST-Aldolase蛋白作为探针蛋白与Actin-His6蛋白液进行GST沉降实验，实验产物进行十二烷基硫酸钠?鄄聚丙烯酰胺凝胶电泳（SDS?鄄PAGE）和蛋白质印迹（Western blotting）分析。 结果 获得了弓形虫aldolase和actin基因序列，构建了相应的原核表达载体。表达并纯化了GST-Aldolase和Actin-His6蛋白。Actin-His6蛋白免疫SD大鼠后获得其抗血清，经抗体亲和纯化柱纯化，获得Actin-His6多克隆抗体。SDS-PAGE和Western blotting 结果显示，GST沉降实验产物中的蛋白条带可被Aldolase-His6多克隆抗体和Actin-His6多克隆抗体识别。 结论 弓形虫醛缩酶与肌动蛋白存在相互作用。

关键词： 刚地弓形虫 醛缩酶 肌动蛋白 GST 沉降技术 蛋白相互作用

Abstract： Objective To identify the protein-protein interaction between aldolase and actin of *Toxoplasma gondii* by GST pull-down. Methods The aldolase and actin genes were obtained from cDNA library by PCR amplification，and subcloned respectively into pGEX-4T-1 and pET30a. The fusion protein GST-Aldolase and Actin-His6 were expressed in *E. coli* upon induction by 1 mmol/L IPTG and then purified with affinity chromatography. Fifteen rats were immunized intradermally with 200 μg Actin-His6 protein per rat at first time to produce the polyclonal antibodies. Then 100 μg Actin-His6 protein per rat on the 2nd-4th immunizations. Rats were immunized for 4 times with 7 days interval. The serum of rats was collected from heart at the fifth day after the final immunization. Glutathione sepharose beads were incubated with GST-Aldolase protein，then incubated with Actin-His6，and bound proteins were eluted using sample buffer. Eluants were resolved by SDS-PAGE and Western blotting. Results The aldolase and actin genes were obtained, and the recombinant plasmid aldolase/pGEX-4T-1，actin/pET30a were successfully constructed. Protein GST-Aldolase and Actin-His6 were expressed and purified *in vitro*. Serum samples were prepared from rats immunized with protein Actin-His6, and polyclonal antibody was purified with affinity chromatography. SDS-PAGE and Western blotting analysis of products from GST pull-down experiment showed that the protein bands on NC membrane were specifically recognized by anti-Aldolase-His6 and anti-Actin-His6 antibody. Conclusion Aldolase interacts with Actin of *Toxoplasma gondii*.

Keywords： *Toxoplasma gondii* Aldolase Actin GST pull-down Protein interaction

Service
把本文推荐给朋友
加入我的书架
加入引用管理器
Email Alert
RSS
作者相关文章
郑斌
尹志奎
何蔼
李卓雅
詹希美

引用本文:
郑斌, 尹志奎, 何蔼, 李卓雅, 詹希美.GST沉降技术验证弓形虫醛缩酶与肌动蛋白的相互作用[J] 中国寄生虫学与寄生虫病杂志, 2011,V29(5): 363-367

ZHENG Bin, YIN Zhi-Kui, HE Ai, LI Zhuo-Ya, DAN Xi-Mei.Protein Interaction between Aldolase and Actin of *Toxoplasma gondii* by GST Pull-down[J] , 2011,V29(5):363-367