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

一种获得高纯度包涵体蛋白的简便方法

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An Easy Way to Purify the Inclusion Body Protein with High Purity from Prokaryotic Expression Cells

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摘要 以日本血吸虫SjBMP基因部分编码序列构建SjBMP?鄄pET-28a(+）重组原核表达质粒，并转化至大肠埃希菌（E. coli）BL21(DE3）进行原核表达。将经过鉴定的目的蛋白rSjBMP以包涵体形式表达的诱导菌样通过Ni^{2+}-NTA Agarose亲和纯化和十二烷基硫酸钠-聚丙烯酰胺凝胶电泳（SDS-PAGE）切胶再纯化。用该纯化蛋白制备免疫血清，用蛋白质印迹（Western blotting）检测其免疫反应性。结果显示，经Ni^{2+}-NTA Agarose亲和纯化和SDS-PAGE切胶再纯化，获得高纯度的目的蛋白，回收率>11.0%。用该纯化蛋白免疫家兔制备免疫血清，获得的血清效价高于1 ： 1 280；Western blotting检测结果表明，用该免疫血清去识别表达的重组蛋白，出现特异的单一条带，表明该纯化蛋白仍保持其抗原性，可用于免疫学相关实验研究。因此，SDS-PAGE切胶纯化后电渗、透析回收是纯化重组包涵体蛋白有效、简便的方法。

关键词： 重组蛋白 原核表达 包涵体 纯化

Abstract： To clone partial ORF of *Sj*BMP and to construct the recombinant SjBMP-pET-28a(+） plasmids, and then to transform them into the competent cells *E. coli* BL21 (DE3）, finally a positive clone was used to be induced by IPTG. The bacterial aggregates with target protein expressed as inclusion bodies were purified by the methods of Ni^{2+}-NTA affinity purification under denaturation condition and SDS-PAGE gel extraction. The purified protein was used to immune rabbits and make antiserum against the *Sj*BMP, and the antiserum were then used to identify the *rSj*BMP by Western blotting. The target protein obtained by Ni^{2+}-NTA Agarose affinity purification was not pure with unspecific proteins, but the protein further purified by SDS-PAGE gel extraction and the dialysis bag horizontal electrophoresis was quite pure, and the recovery rate was more than 11.0%. Meanwhile, Western blotting was used to identify the recombinant *Sj*BMP protein by antiserum, only a specific single strip appeared, which suggested the protein purified by this method kept its anti-genicity, and could be used for common immunological studies. Therefore, the SDS-PAGE gel extraction combining with electroosmosis and dialysis recycling are good and easy to purify the inclusion body proteins.

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刘镕, 钟沁萍, 蒋明森, 董惠芬.一种获得高纯度包涵体蛋白的简便方法[J] 中国寄生虫学与寄生虫病杂志, 2011,V29(5): 399-封三

LIU Rong, Zhong-xin-Ping, Jiang-Ming-Sen, Dong-Hui-Fen.An Easy Way to Purify the Inclusion Body Protein with High Purity from Prokaryotic Expression Cells [J] , 2011,V29(5):399-封三