癌变.畸变.突变 ISSN: 1004-616x CN:44-1063/R 2004; 16(02) :74-77

论著

NGAL蛋白Ni2＋-金属鳌合层析纯化及其鉴定

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收稿日期 2003-4-14 修回日期 2003-7-8 网络版发布日期:

摘要 背景与目的： 通过对新癌基因NGAL的原核融合表达产物进行Ni2＋-金属鳌合层析纯化及其MALDI－TOF－MS 鉴定，最后获得一定丰度与纯度的NGAL蛋白。 材料与方法： 将pDsbA2.0－NGAL融合表达载体转化大肠杆菌，进行IPTG诱导表达，SDS－PAGE分析表达产物的产量与可溶性，然后将表达产物进行N i2＋-金属鳌合层析纯化及其MALDI－TOF－MS鉴定。结果：将pDsbA2.0－NGAL融合表达载体进行IPTG诱导表达，SDS－PAGE分析显示表达的NGAL融合蛋白产量高、可溶性好；对表达的NGAL蛋白进行Ni2＋-金属鳌合层析纯化后其纯度>95 %，MALDI－TOF－MS鉴定结果提示纯化后NGAL蛋白分子量与其理论分子量的误差仅为0.91 ‰ 结论：通过对新癌基因NGAL的原核融合表达产物进行 Ni2＋-金属鳌合层析纯化及其MALDI－TOF－MS鉴定，最后确切获得了一定丰度电泳纯的NGAL蛋白，这为下一步制备其抗体奠定了良好的实验材料。

关键词 NGAL；Ni2＋-金属鳌合层析；6×His；MALDI－TOF－MS

Purification by Ni2＋- Metal Chelate Affinity Chromatography and Identification of NGAL Protein

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Abstract BACKGROUND & AIM: To gain the NGAL protein with definite abundance and purity at last by the novel oncogene NGAL fusion expression in prokarote , purification by Ni2＋- metal chelate affinity chromatography and identification by MALDI－TOF－MS of its expression production. MATERIAL AND METHODS: pDsbA 2.0－NGAL fusion expression vector was transformed to E.coli,induced to express with IPTG and productivity as well as solubility of the expressed production were analyzed via SDS－PAGE. Then expressed production was purified by Ni2＋-metal chelate affinity chromatography and identification by MALDI－TOF－MS. RESULTS: After induced expression of pDsbA2.0－NGAL fusion expression vector with IPTG, SDS－PAGE analysis presented the expressed NGAL fusion protein was high productivity with good solubility. The purity of expressed NGAL protein was more than 95 % after purified by Ni2＋- metal chelate affinity chromatography and identification by MALDI－TOF－MS indicated that the molecular weight error was only 0.91 ‰ between t purified NGAL protein and its theoretical one. CONCLUSION: By the novel oncogene NGAL fusion expression in prokarote, purification by Ni2＋- metal chelate affinity chromatography and identification by MALDI－TOF－MS of its expression production, NGAL protein with definite abundance and electrophoresis purity was truely gained at last, which built up a good experimental material for the preparation of its antibody.

Keywords NGAL； Ni2＋-metal chelate affinity chromatography； 6×His； MALDI－TOF－MS

DOI

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