



## 德国小蠊精氨酸激酶基因的克隆、表达及免疫活性测定

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Cloning, Expression and Purification of Arginine Kinase from *Blattella germanica* and its Immune Activity

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摘要

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**摘要** 目的 克隆德国小蠊精氨酸激酶 (arginine kinase, AK) 全长基因, 表达、纯化重组AK蛋白, 并研究蛋白的免疫反应性。方法 提取德国小蠊总RNA, 反转录为cDNA, 设计特异性引物, PCR克隆德国小蠊AK片段, 将测序正确的目的片段克隆至原核表达载体pET-28a中, 在大肠埃希菌 (*E. coli*) BL21(DE3) 中用异丙基- $\beta$ -D-硫代半乳糖苷 (IPTG) 诱导表达, 通过镍离子亲和和层析纯化目的蛋白。用十二烷基硫酸钠-聚丙烯酰胺凝胶电泳 (SDS-PAGE) 检测目的蛋白表达和纯化结果, 用蛋白质印迹 (Western blotting) 分析重组AK蛋白的免疫反应性。结果 德国小蠊AK基因开放阅读框全长为1 071 bp, 编码356个氨基酸, GenBank登录号为FJ514482。与GenBank中已登录的德国小蠊序列 (登录号为EU429466) 比对, 同源性达97.2%。重组质粒pET-28a-AK在*E. coli* BL21(DE3) 中获得高效表达, 重组蛋白相对分子质量 ( $M_r$ ) 约为45 000, 主要以可溶性形式表达, 经亲和和层析获得目的蛋白。Western blotting分析结果表明, 重组AK蛋白可被过敏性患者血清识别, 免疫反应性良好。结论 成功获得德国小蠊精氨酸激酶全长基因, 重组AK蛋白具有良好的免疫反应性。

**关键词:** 德国小蠊 精氨酸激酶 过敏原 克隆

**Abstract:** Objective To clone and express the arginine kinase (AK) gene of *Blattella germanica* and analyze its immune activity. Methods The cDNA of AK was cloned using specific primers from the total RNA of *Blattella germanica*. The open reading frame (ORF) of AK was cloned into pET-28A vector, and expressed in *Escherichia coli* BL21 (DE3) with IPTG induction. The recombinant protein was purified by Ni<sup>2+</sup> chelating affinity chromatography. The recombinant protein was detected by SDS-PAGE, and its immune activity was analyzed by Western blotting. Results The cloned cDNA ORF sequence (GenBank accession No. FJ514482) contained 1 071 bp and encoded 356 amino acids. Its sequence homology with the published one (GenBank accession No. EU429466) was 97.2% at nucleotide level. The recombinant containing recombinant plasmid pET-28a-AK expressed a soluble protein of AK ( $M_r$  45 000) after being induced with IPTG. The recombinant AK protein was recognized by sera of allergic patients, indicating that the recombinant AK protein has an adequate response activity. Conclusion The AK gene of *Blattella germanica* has been cloned and the recombinant AK protein has been confirmed with immune activity.

**Keywords:** *Blattella germanica* Arginine kinase Allergen Clone

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