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论文

SPAP2基因的克隆、融合表达及分离纯化

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摘要:

本文构建的表达载体pGex-2T-SPAP2CT在大肠杆菌中表达出可融性蛋白, 其分子量为46 000, 经纯化后得到产率 为10%、 纯度大于90%的GST-SPAP2CT蛋白.

关键词: SPAP2基因; 克隆; 融合表达zz')" href="#"> SPAP2基因; 克隆; 融合表达

Cloning, Fusion Expression and Purification of SPAP2 Domain Gene

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Abstract:

SPAP2, a transmembrane protein, is an Ig family receptor containing both ITIMs (immunoreceptor tyrosine-based inhibition motifs) and ITAMs (immunoreceptor tyrosine-based activation motifs). The extracellular portion of SPAP2 contains six immunoglobulin-like domains and its intracellular segment has two ITAMs and two ITIMs. Sequence alignment with the genomic database reveals that the SPAP2 gene contains 16 exons and is localized at chromosome 1g21. SPAP2 is consisted of 734 amino acids, and the intercellular portion of SPAP2 contains 137 amino acids. Tyrosine-phosphorylated SPAP2 is specifically associated with SH2 domain-containing tyrosine kinases and SH2 domain-containing tyrosine 计张小平 phosphatases, which lead to the initiation of signal transduction. SPAP2CT gene was amplified by PCR with SPAP2 full-length DNA as the template and cloned to the pBluescript II KS vector. pGex-2T-SPAP2CT, the expression vector of dissoluble fusion protein, was constructed and transferred into E.coli of DE3-plysS. The fusion protein GST-SPAP2CT was expressed efficiently and purified by FFQ ion exchange chromatography and GSH affinity chromatography. The result indicates that we have constructed steady expression vector pGex-2T-SPAP2CT, which was expressed in E.coli. The molecular weight of the dissoluble fusion protein is 46 000, the productivity of GST-SPAP2CT protein is 10% and the purity is over 90% after the purification.

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