

采用不同疏水相互作用色谱柱从包涵体中快速制备重组人干细胞因子

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Fast preparation of recombinant human stem cell factor from inclusion bodies using different hydrophobic interaction chromatographic columns

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摘要 为了提高重组人干细胞因子(rhSCF)的复性效率,改进了高效疏水相互作用色谱(HPHIC)纯化和复性rhSCF的方法。首先将目标蛋白溶解于8.0 mol/L脲中,然后将rhSCF包涵体的提取液直接进样到不同规格的HPHIC柱进行纯化和复性。优化了固定相配基结构和流动相组成等实验条件,结果表明,本方法可以快速地获得高质量回收率和高生物活性的rhSCF,rhSCF在40 min内即可完成复性与纯化,目标蛋白的纯度在95.5%以上,质量回收率高于49.6%。通过体积排阻色谱和基质辅助激光解吸离子化飞行时间质谱(MALDI-TOF-MS)的分析,确认rhSCF以单体存在。结果进一步证明HPHIC法是同时复性和纯化重组蛋白的有效工具。

关键词: 高效疏水相互作用色谱 重组人干细胞因子 包涵体

Abstract: A method was developed to increase the recovery of recombinant human stem cell factor (rhSCF) from inclusion bodies using high performance hydrophobic interaction chromatography (HPHIC). The target protein was first solubilized in 8.0 mol/L urea solution, and was purified and refolded simultaneously by HPHIC with different chromatographic cakes. Experimental conditions, such as the ligand structures of stationary phase and the composition of mobile phase, were optimized. Under the optimal conditions, high mass recoveries and specific activities of rhSCF were acquired, the purities of rhSCF were above 95.5%, and the mass recoveries of rhSCF were above 49.6%. The final product was also verified as monomer by size exclusion chromatography and matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS). These results provided further evidence that HPHIC is an effective tool in the refolding and purification of recombinant proteins.

Keywords: high performance hydrophobic interaction chromatography (HPHIC) recombinant human stem cell factor inclusion bodies

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