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论文

基于磁珠分离的酶联适配体检测痕量蛋白质的新型比色分析方法

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摘要:

以核酸适配体作为高效专一的识别/传感元件, 构建了一种新型的磁性分离和特异性捕获的检测方法. 两个适配体通过简单的生物素化修饰, 利用其与凝血酶不同位点的高亲和力形成夹心结构, 其中连接适配体的磁珠可捕获蛋白质, 加入另一个适配体及链霉亲和素标记的辣根过氧化物酶后, 通过比色法实现靶蛋白检测. 该法操作简单, 分析时间短, 对凝血酶的线性响应范围为 10~80 nmol/L, 检出限为 10 nmol/L.

关键词: 核酸适配体 凝血酶 磁珠 比色分析

New Magnetic Beads-based Enzyme Linked Aptamer Colorimetric Assay for Trace Amount Protein Detection

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Abstract:

Choosing nucleic acid aptamers as a high efficient and special recognition/sensing element, we developed a novel colorimetric assay based on magnetically separation and special capture to achieve the goal of capturing/tracing target protein. Two different aptamers simply biotinylated, which bound the thrombin in different sites with high affinity, were chosen to develop a sandwich assay. The protein was captured by aptamer-functionalized magnetic beads and detected after the addition of the second biotinylated aptamer and of streptavidin labeled with an enzyme, and the detection of the product generated by enzymatic reaction was achieved by colorimetric assay. This magnetic beads based enzyme linked aptamer assay was capable of capturing thrombin with high specificity, didn't be affected by other interfering proteins in complex matrix such as human serum albumin and bovine hemoglobin. The detection of thrombin in serum could be carried out with naked eyes, without the need of expensive analytical instruments and more assay time. A linear range from 10 to 80 nmol/L is obtained with a detection limit of 10 nmol/L for thrombin.

Keywords: Aptamer Thrombin Magnetic bead Colorimetric

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