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论著

TRAP 结合液体闪烁计数法半定量检测端粒酶活性

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摘要 本文介绍一种端粒酶检测方法,该方法是将TRAP 法在PCR 过程中引入3 H2dCTP ,结合液闪计数cpm 半定量分析端粒酶活性。应用该方法检测端粒酶阳性的CNE2 细胞和部分组织标本及RNase A 或加热预处理后的对照标本,并与TRAP 法相比较,结果显示CNE2 细胞端粒酶阳性,10 个CNE2 细胞中仍可检到端粒酶,组织样品的端粒酶与文献值基本一致;放射性活性计数(cpm) 与CNE2 细胞抽提液中端粒酶活性具有良好的线性关系;用RNase A 或加热处理后标本为阴性,cpm 值接近阴性对照;批内和批间变异度分别为11. 68 %和20199 %。该方法不需使用聚丙烯酰胺凝胶电泳(PAGE) 和放射自显影,简便快速,当天可观察结果,并具有灵敏度高、特异性和批内重复性好之特点。

关键词 端粒酶 定量分析 端粒重复序列扩增法 液体闪烁计数

QUANTITATIVE DETECTION OF TELOMERASE ACTIVITY BY COMBINATION OF TRAP METHOD AND SCINTILLATION COUNT ASSAY

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Abstract This study developed a semi2quantitative method of telomerase activity. The method was based on the combination of TRAP assay and scintillation count assay (3 H2dCTP incorporation) . It was used to quantify telomerase activity in CNE2 cells and some tissue specimens. RNase2pret reated or heat2t reated cells were used as cont rols. The result s demonst rated that telomerase activity measured by this method was positive in CNE2 cells , and it could be clearly detected with as few as 10 cells. There was a linear correlation between the radioactive count and the telomerase activity. The telomerase activity of some tissue specimens were accordant with reported data. Telomerase activity of RNase2pret reated or heatt reated cells was negative , their radioactive counts were almost the same as lysis buffer cont rol. The variations within group and between groups were 11. 68 % and 20.99 % , respectively. This method was f ree of PAGE and radioautography , and hence simple and fast . The results could be obtained with one day. It showed high sensitivity , good specificity and repeatability.

Keywords Telomerase Quantitative Analysis Telomeric Repeat Amplification Protocol Scintillation Count Assay

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