

### DNA末端定量及其应用的研究

### Quantitation of DNA Breaks and Application of the Assay

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英文关键词: [DNA break](#) [saturation label](#) [terminal deoxynucleotidyl transferase\(TdT\)](#)

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中文摘要:

为定量DNA断裂末端评价DNA的降解程度,以饱和标记(TdT)法定量DNA末端最大标记量( $L_{max}$ );并用流式细胞分析(FCA)、原位DNA末端标记(TUNEL)和琼脂糖电泳检测或标记DNA降解片段.结果发现: a. TdT法最低检测限5 ng DNA,线性范围为5~5 000 ng,较检测DNA降解片段的琼脂糖电泳敏感200倍以上; b. 地塞米松(DEX)诱导淋巴瘤(Raji)细胞凋亡产生的 $L_{max}$ 呈DEX剂量与诱导时间依赖性增加; c. 自发性高血压大鼠(SHR)心肌的 $L_{max}$ 呈年龄依赖性增加并与正常对照鼠(WKY)相比差异非常显著( $P<0.01$ ); d. TdT法定量 $L_{max}$ 与FCA、TUNEL或电泳分析的相关性良好( $r>0.98$ ). TdT法定量 $L_{max}$ 敏感、特异、准确,可应用于分子生物学、细胞生物学,尤其是细胞凋亡动力学DNA降解评价的定量研究.

英文摘要:

To quantitate DNA breaks, a method based on saturation labeling 3' -ends of DNA fragments with  $\alpha$ -<sup>32</sup>P dCTP in the presence of 2', 3' -dideoxy-cytidine-5' -triphosphate (ddCTP) by terminal deoxynucleotidyl transferase (TdT) was developed. The saturation labeling of 3' -ends of DNA fragments was performed by adding different concentrations of  $\alpha$ -<sup>32</sup>P dCTP to a DNA sample, from which a maximal labeling ( $L_{max}$ ) and a kinetic parameter ( $K_m$ ) of the TdT reaction were calculated. Results were confirmed by agarose gel electrophoresis, fluorescein-dUTP and exogenous terminal deoxynucleotidyl transferase(TUNEL), flow cytometric analysis (FCA). The method mentioned above requires as little as 5 ng of DNA, increases in the sensitivity of DNA fragments detection by at least 200-fold relative to the widely used agarose gel electrophoresis, and the linearity of the assay is about 5~5 000 ng DNA. The application of the method in the apoptosis study showed that(1) a time- and dose-dependent increase in the number of DNA strand breaks in apoptotic Raji lymphoma lymphocytes induced by dexamethasone, and (2) age-dependent increase in the number of DNA strand breaks occurred in the cardiac tissues of spontaneously hypertensive rats (SHR) compared with that of normal control rats (WKY). Results of the assay were confirmed by the DNA ladder pattern exhibited after electrophoresis, fluorescein-dUTP and exogenous terminal deoxynucleotidyl transferase(TUNEL), flow cytometric analysis(FCA) ( $r>0.98$ ). It is a quantitative, simple, sensitive, specific and useful assay for assessing DNA degradation in molecular and cell biology especially in apoptosis research.

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