

Apo ssDNA

An antibody assay to detect DNA damage (single stranded DNA) in Apoptotic Cells

Key Benefits

- More reliable than TUNEL assay, no false positive signals.
- Readout - Flow cytometry, 96 well plate reader, Fluorescence microscope
- Yields both quantitative and qualitative results. Gives a strong positive signal.
- Ease Of Use: Get results in less than 60 minutes.
- No need to run DNA ladder assays to detect DNA damage.

Introduction

A widely used cytochemical technique for the evaluation of DNA damage associated with apoptosis is the terminal deoxynucleotidyl transferase-mediated in situ end labeling or TUNEL assay. However the TUNEL assay has its drawbacks in that, false positive staining makes the assay unreliable as a marker for apoptosis (see figure 1) ¹⁻⁵. A more universal and specific marker for apoptosis associated DNA damage is the morphological changes in nuclei that reflect chromatin condensation into compact masses ⁶⁻⁷.

Further biochemical and cytochemical studies have demonstrated the increased susceptibility of apoptotic DNA to thermal denaturation. Analysis of nuclei by scanning calorimetry to detect thermal induced DNA denaturation and analysis of DNA fragmentation by electrophoresis have shown that intact apoptotic DNA is susceptible to denaturation at lower temperatures than that of non-apoptotic cells ⁸.

Assay Principle

Innovative Research introduces Apo ssDNA, an antibody based assay to detect DNA damage (single stranded DNA: ssDNA) in Apoptotic Cells.

The assay utilizes an antibody generated against ssDNA. This antibody recognizes large stretches of heat-denatured ssDNA only in apoptotic cells ⁹⁻¹²

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Figure 1 (A) Figure 1 (B)

Monoclonal anti ssDNA TUNEL Assay

Fig 1 (A). MAb to ssDNA staining of apoptotic but not of necrotic cells. Fluorescence distributions of MDA-468 cells heated and stained with MAb F7-26 were generated on a flow cytometer. Apoptosis was induced by staurosporine and necrosis was induced by sodium azide, saponin, or hyperthermia. Note that apoptotic cells are intensely stained with the MAb, whereas the fluorescence profiles of necrotic and control cells are similar.

Fig1.(B). TUNEL staining of apoptotic and necrotic cells. Fluorescence distributions of MDA-468 cells stained with TUNEL were generated on a flow cytometer. Apoptosis was induced by staurosporine and necrosis was induced by sodium azide, saponin, or hyperthermia. Note that cells at early stage of apoptosis (staurosporine 3 hr) are weakly stained, whereas late apoptotic cells (staurosporine 5 hr) and necrotic cells are intensely stained by TUNEL .

Kit Content

1. Mouse anti single stranded DNA
2. Anti mouse IgM FITC labeled
3. Fixative
4. 10 X Wash Buffer
5. 1X DNA Denaturing Buffer