

ab66108

***In situ* Direct DNA
Fragmentation (TUNEL)
Assay Kit**

Instructions for Use

For the rapid, sensitive and accurate measurement of apoptosis in various samples.

This product is for research use only and is not intended for diagnostic use.

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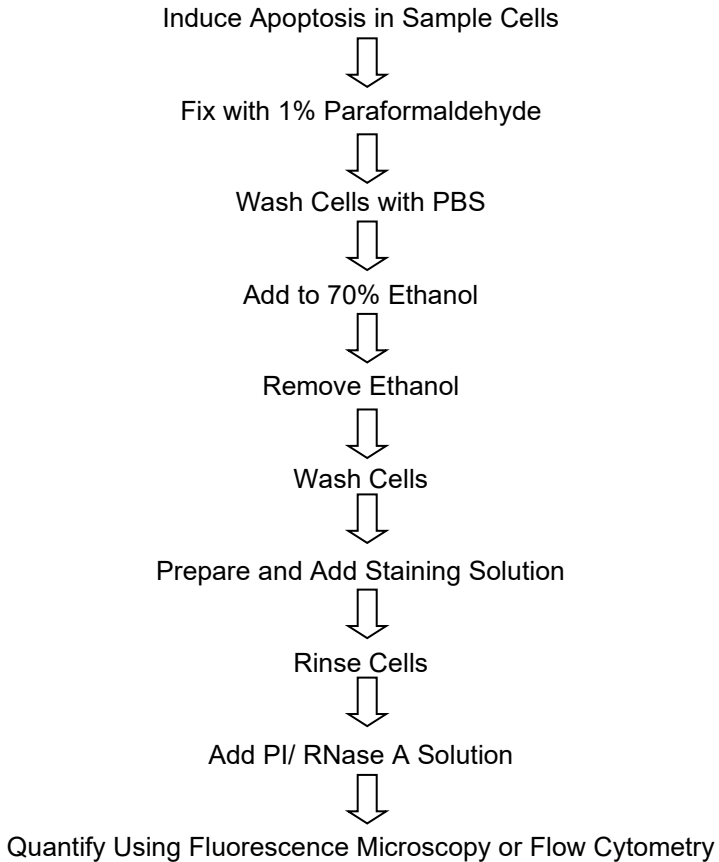
1. Overview

Internucleosomal DNA fragmentation is a hallmark of apoptosis in mammalian cells.

Abcam's *In situ* Direct DNA Fragmentation (TUNEL) Assay Kit provides complete components including positive and negative control cells for conveniently detecting DNA fragmentation by fluorescence microscopy or flow cytometry. The TUNEL-based detection kit utilizes terminal deoxynucleotidyl transferase (TdT) to catalyze incorporation of fluorescein-12-dUTP at the free 3'-hydroxyl ends of the fragmented DNA. The fluorescein-labeled DNA can then be observed by fluorescence microscopy or analyzed by flow cytometry.

Detection method – Flow cytometry (Ex/Em = 488/520 nm for FITC, and 488/623 nm for PI).

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity	Storage Temp.
Positive Control Cells**	5 mL	-20°C
Negative Control Cells**	5 mL	-20°C
Wash Buffer	100 mL	+4°C
Reaction Buffer	0.5 mL	+4°C
TdT Enzyme	38 µL	-20°C
FITC-dUTP	0.40 mL	-20°C
Rinse Buffer	100 mL	+4°C
PI/RNase Staining Buffer	25 mL	+4°C

* Store components separately according Table A.

**Positive control: Human lymphoma cell (apoptosis induced by camptothecin)

Negative control: Human lymphoma cell

The concentration of cells in control: 1×10^6 cells/mL.

B. Additional Materials Required

- Microcentrifuge
- PBS
- Pipettes and pipette tips
- Flow Cytometer
- 1% Paraformaldehyde
- 70% Ethanol
- Orbital Shaker

4. Assay Protocol

1. Cell Fixation:

a) Induce apoptosis by desired methods. Concurrently incubate a control culture *without* induction.

b) Suspension cells: Collect $1-5 \times 10^6$ cells by centrifugation at 300 x g. Re-suspend in 0.5 ml of PBS.

Adherent cells: Trypsinize cells in trypsin/EDTA for 1-2 min, stop trypsinization by adding culture medium and pellet by centrifugation at 300 x g. Re-suspend in 0.5 ml of PBS.

Δ Note: take cell supernatant from adherent cell culture and centrifuge together with trypsinized cells. Fix the cells by adding 5 ml of 1% (w/v) paraformaldehyde in PBS and place on ice for 15 min.

c) Centrifuge the cells for 5 min at 300 x g and discard the supernatant.

d) Wash cells in 5 ml of PBS and pellet the cells by centrifugation. Repeat the wash and centrifugation step one more time.

e) Re-suspend the cells in 0.5 ml of PBS.

f) Add the cells to 5 ml of ice-cold 70% (v/v) ethanol. Let cells stand for a minimum of 30 min on ice or in the freezer.

- g)** Store the cells in 70% (v/v) ethanol at -20°C until use. Cells can be stored at -20°C for several days before use.

2. Assay Procedure:

The procedures can be used for both control cells and your testing cells.

- a) Re-suspend the fixed cells by swirling the vials. Remove 1 ml aliquots of the cell suspension ($\sim 1 \times 10^6$ cells per ml) and place in 12 x 75 mm tubes. Centrifuge (300 x g) for 5 min and carefully remove the ethanol by aspiration.
- b) Re-suspend the cells with 1 ml of Wash Buffer. Centrifuge as before and remove supernatant carefully by aspiration.
- c) Repeat the washing step (step b) one more time.
- d) Re-suspend in 50 μ l of the Staining Solution prepared as below:

DNA Labeling Solution	1 assay	10 assays
Reaction Buffer	10 μ L	100 μ L
TdT Enzyme	0.75 μ L	7.5 μ L
FITC-dUTP	8 μ L	80 μ L
ddH ₂ O	32.25 μ L	322.5 μ L
Total Volume	51 μ L	510 μ L

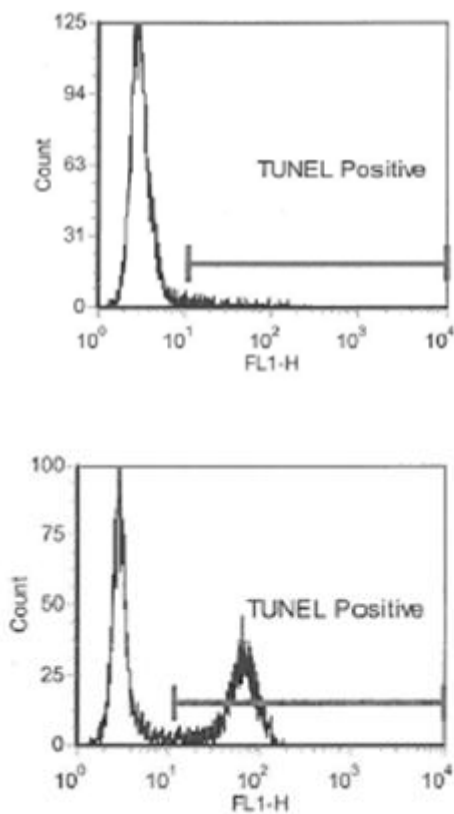
- e) Incubate the cells in the Staining Solution for 60 min at 37°C. Shake cells every 15 min to re-suspend.

- f)** Add 1 ml of Rinse Buffer to each tube and centrifuge for 5 min. Remove supernatant by aspiration.
- g)** Repeat the rinsing step one more time (step f).
- h)** Re-suspend the cell pellet in 0.5 ml of Propidium Iodide/RNase A Solution.
- i)** Incubate the cells in the dark for 30 min at room temperature.
- j)** Observe the cells by fluorescence microscopy (apoptotic cells show green staining over an orange-red PI counter-staining). To transfer the cells to slides take 50-100ul of cells and put it on the slide, invert a coverslip on it and seal with nail-polish. Poly-lysine treated slides are better.

Or

Analyze by flow cytometry (Ex/Em = 488/520 nm for FITC, and 488/623 nm for PI). Cells should be analyzed within 3 hours of staining.

Example of Images



Top example: Negative Control cells

Bottom example: Positive Control cells

Technical Support

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