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.

Version 7b Last Updated 11 January 2021

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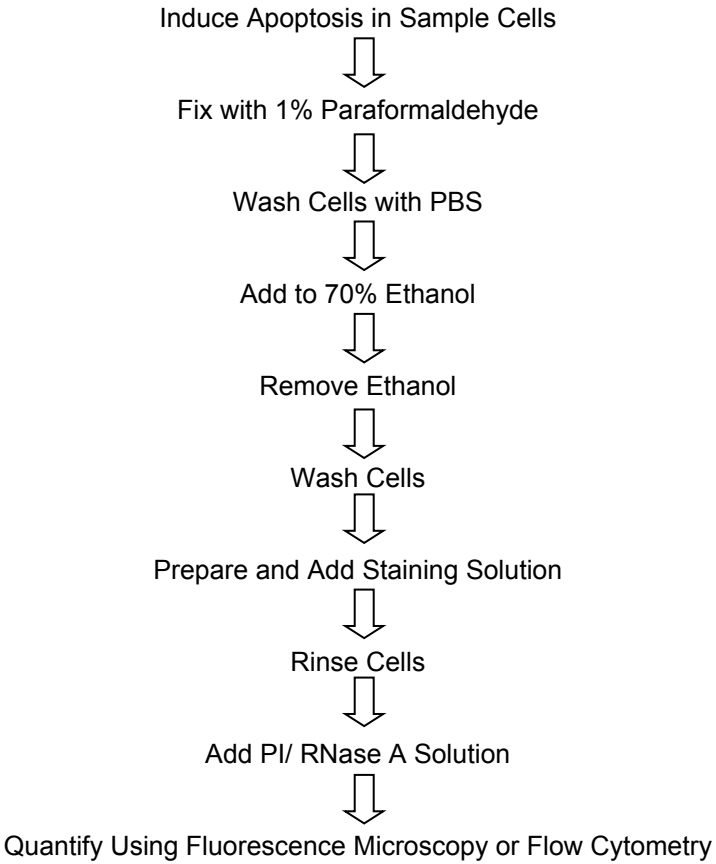
1. Overview

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. Shelf life is 1 year from the date of the product shipment, under proper storage conditions.

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

Negative control: Human lymphoma cell

The concentration of cells in control: 1 x 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 x 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 (~1 x 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 µl of the Staining Solution prepared as below:

DNA Labeling Solution	**1 assay**	**10 assays**
Reaction Buffer	10 µL	100 µL
TdT Enzyme	0.75 µL	7.5 µL
FITC-dUTP	8 µL	80 µL
ddH_2O	32.25 µL	322.5 µL
Total Volume	51 µL	510 µL

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.

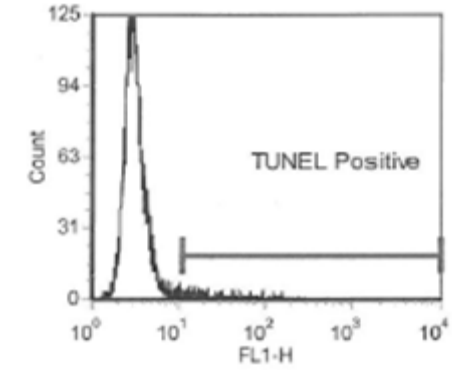
i) Incubate the cells in the dark for 30 min at room temperature.

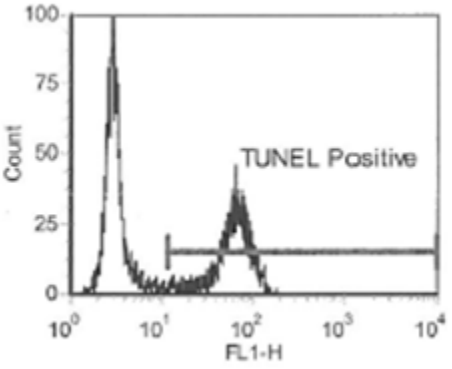
j) Observe the cells by fluorescence microscopy (apoptotic cells show green staining over an orange-red PI counter-staining). To transfer the cells to slides take 50-100ul of cells and put it on the slide, invert a coverslip on it and seal with nail-polish. Poly-lysine treated slides are better.

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

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