Apo-Direct Apoptosis Detection Kit

Catalog Number: 88-6611
RUO: For Research Use Only. Not for use in diagnostic procedures.

Product Information

Contents: Apo-Direct Apoptosis Detection Kit
REF **Catalog Number:** 88-6611

Temperature Limitation: Refer to vials for proper storage conditions.
LOT **Batch Code:** Refer to vial
Use By: Refer to vial

Description

The APO-DIRECT™ Kit is a 2-color staining method for labeling DNA breaks and total cellular DNA to detect apoptotic cells by flow cytometry. The kit contains the instructions and reagents required for measuring apoptosis in cells including positive and negative control cells for assessing reagent performance; washing, reaction and rinsing buffers for processing individual steps in the assay; terminal deoxynucleotidyl transferase enzyme (TdT), Fluorescein-deoxyuridine triphosphate and propidium iodide/RNase A solution for counterstaining the total DNA.

One of the most easily measured features of apoptotic cells is the break-up of the genomic DNA by cellular nucleases. These DNA fragments can be extracted from apoptotic cells and result in the appearance of DNA laddering when the DNA is analyzed by agarose gel electrophoresis. The DNA of non-apoptotic cells that remains largely intact does not display this laddering on agarose gels during electrophoresis. The large number of DNA fragments appearing in apoptotic cells results in a multitude of 3'-hydroxyl termini in the DNA. This property can be used to identify apoptotic cells by labeling the 3'-hydroxyl ends with directly conjugated fluorescein- deoxyuridine triphosphate nucleotides (FITC-dUTP). The enzyme terminal deoxynucleotidyl transferase (TdT) catalyzes a template-independent addition of deoxyribonucleoside triphosphates to the 3'-hydroxyl ends of double- or single-stranded DNA with either blunt, recessed or overhanging ends. A substantial number of these sites are available in apoptotic cells providing the basis for the method utilized in the APO-DIRECT™ Kit. Non-apoptotic cells do not incorporate significant amounts of the FITC-dUTP due to the lack of exposed 3'-hydroxyl DNA ends.

Sufficient reagents are provided to process 50 cell suspensions including 5 mL positive and 5 mL negative control cell suspensions of approximately $1x10^6$ cells per mL in 70% (v/v) ethanol.

Tel: 888.999.1371 or 858.642.2058 • Fax: 858.642.2046 • thermofisher.com/ebioscience • info@ebioscience.com

invitrogen

Apo-Direct™ Staining Protocols

Introduction

The following protocol describes the method for measuring apoptosis in the positive and negative control cells that are provided in the Apo-Direct™ Kit. The same procedure should be employed for measuring apoptosis in the cell specimens supplied by the researcher.

General Protocol: Apo-Direct™

Experimental Procedure

1. Resuspend the positive (brown cap) and negative (natural cap) control cells by swirling the vials. Remove 1 mL aliquots of the control cell suspensions (approximately 1x10^6 cells per 1 mL) and place in 12x75 mm flow cytometry centrifuge tubes. Centrifuge the control cell suspensions for 5 minutes at 300 x*g*, then remove the 70% (v/v) ethanol by aspiration, being careful to not disturb the cell pellet.
2. Resuspend each tube of control cells with 1 mL of Wash Buffer (blue cap). Centrifuge as before and remove the supernatant by aspiration.
3. Repeat the Wash Buffer treatment (step 2).
4. Resuspend each tube of the control cell pellets in 50 µL of the DNA Labeling Solution (prepared as described below).

DNA LABELING SOLUTION	1 ASSAY	6 ASSAYS (2 controls+ 4 samples)	12 ASSAYS (2 controls+ 10 samples)
TdT Reaction Buffer (green cap)	10.00 µL	60.00 µL	120.00 µL
TdT Enzyme (yellow cap)	0.75 µL	4.5 µL	9.00 µL
Fluorescein-dUTP (orange cap)	8.00 µL	48.00 µL	96.00 µL
Distilled H_2O	32.00 µL	192.0 µL	384.00 µL
Total Volume	51.75 µL	304.5 µL	609.00 µL

Note: The appropriate volume of Staining Solution to prepare for a variable number of assays is based upon multiples of the component volumes combined for 1 Assay. Mix only enough Staining Solution to complete the number of assays prepared per session. The Staining Solution is active for approximately 24 hours.

5. Incubate the cells in the DNA Labeling Solution for 60 minutes at 37°C in a temperature-controlled bath. Shake cells every 15 minutes to resuspend.

   Note: The staining reaction can also be carried out overnight at 22-24°C for the control cells. For samples other than the control cells provided in the kit, incubation times at 37°C may need to be adjusted to longer or shorter periods depending on the characteristics of the cells supplied by the researcher.
6. At the end of the incubation time add 1.0 mL of Rinse Buffer (red cap) to each tube and centrifuge each tube for 5 minutes at 300 xg. Remove the supernatant by aspiration.
7. Repeat the cell rinsing as in step 6 and remove the supernatant by aspiration.
8. Resuspend the cell pellet in 0.5 mL of the Propidium Iodide/RNase A Solution (amber bottle).
9. Incubate the cells in the dark for 30 minutes at room temperature.
10. Analyze the cells in the Propidium Iodide/RNase A Solution by flow cytometry.
11. Analyze the cells within 3 hours of staining.

Cell Fixation Procedure for Apo-Direct™ Assay:

Note: Cell fixation using paraformaldehyde is a required step in the Apo-Direct™ assay. The following cell fixation procedure is a suggested method. Variables such as cell origin and growth conditions can affect the results. The fixation conditions provided below should be considered as guidelines. Additional experimentation may be required to obtain results comparable to the control cells provide with this kit. The positive and negative control cells provided in the Apo-Direct™ Kit are already fixed.

1. Suspend 1-2x10^6 cells in 0.5 mL of phosphate buffered saline (PBS) (10 mM sodium phosphate pH 7.2, 150 mM sodium chloride).
2. Add the cell suspension into 5 mL of 1% (w/v) paraformaldehyde in PBS and incubate on ice for 15 minutes.
3. Centrifuge cells for 5 minutes at 300 x*g* and discard the supernatant.
4. Wash the cells in 5 mL of PBS then pellet the cells by centrifugation. Discard the supernatant.
5. Repeat the wash and centrifugation.

6. Resuspend the cells in 0.5 mL of PBS.
7. Adjust the cell concentration to 1-2x10^6 cells/mL in 70% (v/v) ice cold ethanol and incubate the cells on ice or at -20°C for a minimum of 30 minutes before staining.

   Note: In some biological systems, storage of the cells at -20°C in 70% (v/v) ethanol for at least 12-18 hours prior to staining for apoptosis detection yields the best results.
8. Store cells in 70% (v/v) ethanol at -20°C until use. Cells can be stored at -20°C several days before use.

Documentation and support

Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **thermofisher.com/support**.

Corporate entity: Life Technologies | Carlsbad, CA 92008 USA | Toll Free in USA 1.800.955.6288