APO-BrdU™ TUNEL Assay Kit

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

A landmark of cellular self-destruction by apoptosis is the activation of nucleases that eventually degrade the nuclear DNA into fragments of approximately 200 base pairs in length. Detection of these DNA fragments is relatively straightforward, making this assay among the most reliable methods for identifying apoptotic cells. The APO-BrdU™ TUNEL Assay Kit detects the DNA fragmentation of apoptotic cells by exploiting the fact that the DNA breaks expose a large number of 3′-hydroxyl ends. These hydroxyl groups can then serve as starting points for terminal deoxynucleotidyl transferase (TdT), which adds deoxyribonucleotides in a template-independent fashion. Addition of the deoxythymidine analog 5-bromo-2′-deoxyuridine 5′-triphosphate (BrdUTP) to the TdT reaction serves to label the break sites. Once incorporated into the DNA, BrdU can be detected by an anti-BrdU antibody using standard immunohistochemical techniques. This method of labeling DNA breaks is referred to as Terminal Deoxynucleotide **T**ransferase d**U**TP **N**ick **E**nd **L**abeling, or TUNEL.

The APO-BrdU™ TUNEL Assay Kit provides the materials necessary to label DNA strand breaks for the detection of apoptotic cells, as well as fixed samples of positive and negative control cells for assessing assay performance. Complete protocols for flow cytometry applications are also included, although the kit may be adapted for use with fluorescence microscopy as well. Final detection of BrdU incorporation at DNA break sites is achieved through an Alexa Fluor™ 488 dye–labeled anti-BrdU antibody. The Alexa Fluor™ 488 dye, which has excitation and emission maxima similar to those of fluorescein, produces fluorescent conjugates that are brighter and more photostable than commonly used fluorescein conjugates. The kit also includes propidium iodide to determine the total cellular DNA content.

Contents and storage

Component	Amount	Storage[1,2]
Bag 1 of 2		
Positive control cells, fixed human lymphoma cell line[3] (Component A, brown cap)	5 mL	–20°C Protect from light
Negative control cells, fixed human lymphoma cell line[3] (Component B, white cap)	5 mL	
Terminal deoxynucleotidyl transferase (Component C, yellow cap)	45 µL	
5-Bromo-2'-deoxyuridine 5'-triphosphate (BrdUTP) (Component D, violet cap)	480 µL	

Component	Amount	Storage[1,2]
Bag 2 of 2		
Anti-BrdU mouse monoclonal antibody PRB-1, Alexa Fluor™ 488 conjugate (Component E, orange cap)	350 µL	2°C to 8°C Protect from light DO NOT FREEZE
Propidium iodide/RNase A staining buffer (Component F, amber bottle)	30 mL	
Reaction buffer (Component G, green cap)	0.6 mL	
Wash buffer (Component H, blue cap)	120 mL	
Rinse buffer (Component I, red cap)	120 mL	
Number of assays: 60 assays of 1 mL samples containing 1×10^6 to 2×10^6 cells/mL		
Approximate fluorescence excitation and emission maxima: 495/519 nm for the Alexa Fluor™ 488 labeled monoclonal antibody.		

[1] Each module can be stored under the conditions listed. For optimal storage conditions of individual components, refer to the vial labels.
[2] When stored as directed, the kit components are stable for at least 3 months.
[3] The positive and negative control cells are suspensions of 1×10^6 to 2×10^6 cells/mL in 70% ethanol.

Perform the TUNEL assay

The following procedure was designed for use in flow cytometry applications, but is adaptable to fluorescence microscopy. This protocol requires that cells be in suspension; adherent cell lines may be used but must be trypsinized after induction of apoptosis.

Note: For microscopy applications, it is recommended that the cells be deposited onto slides after the antibody staining step, but prior to the propidium iodide/RNase treatment. Cells that have undergone apoptosis should brightly fluoresce when viewed with filter sets appropriate for fluorescein.

Note: For adherent cell lines, detached cells present in the supernatant have a higher probability of being apoptotic than do cells that have remained adherent. Detached cells should be collected prior to trypsinization of the adherent cell layer.

1 Prepare and fix cells

Cell fixation using paraformaldehyde is a required step in the APO-BrdU™ assay. The following cell fixation procedure is a suggested method. Variables such as cell origin and growth conditions can affect the results, so the fixation conditions described below should be considered as guidelines. The positive and negative control cells provided in the APO-BrdU™ TUNEL Assay Kit are already fixed.

Note: Cell fixation is an important step in analyzing apoptotic samples. Unfixed cells may lose smaller DNA fragments, leading to lower signals. Alternate fixation methods may be necessary to fully exploit some cell systems.

1.1. Induce apoptosis in cells using the desired method. Although negative control cells are provided with the kit, it may be desirable to prepare a negative control sample using the cell line of interest by incubating cells in the absence of inducing agent.

1.2. Suspend 1×10^6 to 2×10^6 cells in 0.5 mL of phosphate-buffered saline (PBS).

1.3. Add the cell suspension into 5 mL of 1% (w/v) paraformaldehyde in PBS and place on ice for 15 minutes.

1.4. Centrifuge the cells for 5 minutes at 300 × *g* and discard the supernatant.

1.5. Wash the cells in 5 mL of PBS then pellet the cells by centrifugation. Repeat step 1.5.

1.6. Resuspend the cells in 0.5 mL of PBS.

1.7. Add the cells to 5 mL of ice-cold 70% (v/v) ethanol. Let the cells stand for a minimum of 30 minutes on ice or in a –20°C freezer. In some biological systems, storage of the cells at –20°C in 70% (v/v) ethanol for at least 12–18 hours prior to performing the TUNEL assay yields the best results. Cells can be stored at –20°C for several days before use.

2 Detect apoptotic cells

The following protocol describes the method for measuring apoptosis in the positive and negative control cells that are provided in the APO-BrdU™ TUNEL Assay Kit. The same procedure should be employed for measuring apoptosis in the experimental samples.

2.1. Resuspend the positive (brown cap) and negative (white cap) control cells by swirling the vials. Remove 1 mL aliquots of the control cell suspensions (approximately 1×10^6 cells/mL) and place in 12 × 75-mm flow cytometry centrifuge tubes. Centrifuge (300 × *g*) the control cell suspensions for 5 minutes and remove the 70% (v/v) ethanol by aspiration, being careful to not disturb the cell pellet.

Note: To minimize cell loss, use a single 12 × 75-mm test tube for each assay. Use of sequential tubes can result in significant cell loss during the assay due to cells sticking to the sides of the tube.

2.2. Resuspend the control cells of each tube with 1 mL of wash buffer (blue cap). Centrifuge for 5 minutes at 300 × *g* and remove the supernatants by aspiration. Repeat step 2.2.

2.3. Prepare a DNA-labeling solution; a total volume of 50 µL is required for each sample. Mix 10 µL of reaction buffer (green cap), 0.75 µL of TdT enzyme (yellow cap), 8.0 µL of BrdUTP (violet cap) and 31.25 µL of dH_2O. For additional samples, the volumes may be scaled up accordingly, but mix only enough DNA-labeling solution to complete the number of assays prepared per session. The DNA-labeling solution is active for approximately 24 hours.

2.4. Resuspend the control cell pellets of each tube in 50 µL of the DNA-labeling solution (prepared in step 2.3).

2.5. Incubate the cells in the DNA-labeling solution for 60 minutes at 37°C in a temperature-controlled bath. Shake the samples every 15 minutes to keep the cells in suspension. 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 experimental samples. The DNA-labeling reaction for the control cells can also be carried out at 22–24°C overnight.

2.6. At the end of the incubation time, add 1.0 mL of rinse buffer (red cap) to each tube and centrifuge at 300 × *g* for 5 minutes. Remove the supernatants by aspiration.

2.7. Repeat the cell rinsing (as in step 2.6) with 1.0 mL of rinse buffer (red cap). Centrifuge the samples at 300 × *g* and remove the supernatants by aspiration.

2.8. Prepare 100 µL of antibody staining solution for each sample by mixing 5.0 µL of the Alexa Fluor™ 488 dye–labeled anti-BrdU antibody (orange cap) with 95 µL of rinse buffer (red cap). Prepare only enough antibody staining solution to complete the number of assays prepared per session.

2.9. Resuspend the cell pellets in 100 µL of the antibody solution prepared in step 2.8. Incubate the cells in this solution for 30 minutes at room temperature. Protect the samples from light during the incubation.

2.10. *(Optional)* Add 0.5 mL of the propidium iodide/RNase A staining buffer (amber bottle) to each sample. Incubate the cells for an additional 30 minutes at room temperature. Protect the samples from light during the incubation.

Note: If the DNA cell cycle information is not required, it is not necessary to add the PI/RNase A staining buffer to each tube.

2.11. Analyze the samples by flow cytometry. It is recommended that the samples be analyzed within 3 hours of completing the staining procedure.

Note: If signal intensity is low, the incubation time for the DNA-labeling reaction (step 2.5) can be extended. Labeling times of up to 4 hours at 37°C may be required for some systems.

Note: Occasionally a mirror image population of lower intensity cells is observed in the flow cytometry dual parameter display. This population arises when some cells become stuck to the side of the test tube and are not fully exposed to the DNA-labeling solution. Make sure all cells are properly suspended at the beginning of the labeling reaction.

Limited product warranty

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Revision history: Pub. No. MAN0002269

Revision	Date	Description
B.0	16 August 2022	The format and content were updated.
A.0	30 November 2017	Documents were rebranded. Legal and regulatory language was updated.
1.0	16 February 2007	Basis for this revision.