Apoptosis Assay Cocktail

Cat #: KTD102-EN Size: 1 kit

	Apoptosis Assay Cocktail		
REF	**Cat #:** KTD102-EN	LOT	**Lot #:** Refer to product label
	Applications: Flow cytometry and fluorescence detection of tissue and cell samples		
	Note: Stored at -20℃ for 6 months, and storing according to the storage conditions of each component after the package is opened		

Assay Principle

Apoptotic cells produces a number of important changes, including cell shrinkage, cytoplasmic agglutination, DNA disruption, and formation of membrane vesicles, and lead to the separation of cell components to form apoptotic bodies, which are eventually phagocytic by macrophages or parenchyma cells[1]. Vermes was the first researcher to use Annexin V staining to specifically identify phosphatidylserine (PS) on the ectropion cell membrane, and the Annexin V assay is considered the most classical method by researchers worldwide[2]. In addition, DNA fragmentation is an important characteristic in the late stage of apoptosis, and TUNEL method based on DNA fragmentation detection is regarded as another gold standard for apoptosis research[3]. Based on the views of domestic and foreign experts in the field of apoptosis research as well as a large number of statistical analysis of literature, Abbkine has developed a cocktail set to detect different stages of early and late apoptosis simultaneously based on the new generation of AbFluor™ fluorescent staining and high efficiency recombinant protein technology. It's suitable for different samples such as tissues and cells. High sensitivity and universal apoptosis detection kit can effectively distinguish apoptosis from necrosis. Meanwhile, aiming at the non-standardization of apoptosis detection and results, we developed a patent positive control substance for apoptosis detection, which was combined into the Apoptosis Assay Cocktail. This product can be used as a standardized product for cell apoptosis research and can meet the needs of most cell apoptosis detection. It is the best choice for cell apoptosis detection. Details of the Apoptosis Assay Cocktail are as follows:

Components	Expatiation for kit	Size	storage condition
Annexin V-AbFluor™ 488 Apoptosis Detection Kit-40T	Annexin V Binding Buffer (5×)	4 mL	4℃
	Annexin V-AbFluor™ 488	200 μL	4℃, protected from light
	Propidium Iodide (PI)	80 μL	4℃, protected from light
TUNEL Apoptosis Detection Kit (Green Fluorescence)-20T	TdT Enzyme	20 μL	-20℃
	Equilibration Buffer (5×)	600 μL	-20℃
	Label Mix Green	100 μL	-20℃, protected from light
	Triton X-100 (100%)	100 μL	4℃
	BSA Working Solution	3 mL	-20℃

	DAPI (500×)	8 μL	-20℃, protected from light
Apoptosis Positive Control-5T	Apoptosis Inducer A	5 μL	-20℃
	Apoptosis Inducer B	5 μL	-20℃

Reagent Preparation

Annexin V Binding Buffer (1×): Prepare 1×Annexin V Binding Buffer by dilute 5×Annexin V Binding Buffer with deionized water.
Equilibration Buffer (1×): Prepare 1×Equilibration Buffer by dilute 5×Equilibration Buffer with deionized water.

Assay Procedure

Ⅰ. Positive cell apoptosis induction

1. For cultured cells to be induced apoptosis, the Apoptosis Inducer A or Apoptosis Inducer B were added into the culture medium according to the volume ratio of 1:1000-1:3000 (Apoptosis Inducer A and Apoptosis Inducer B can be added into the culture medium together).
2. Cell apoptosis was observed after 4, 8, 12, 16 or 24 h. For the most part, after 16-24 h, obvious changes in cell morphology can be seen under the light microscope, which can be used to observe apoptosis staining (It is recommended to adjust the induction time for different cells).

Ⅱ. Detection cell apoptosis by TUNEL assay

A. Sample Preparation

1. For adherent cells (Analysis by Fluorescence microscope)

(1) Grown in a 96-well microplate culture for at least 24 h. Induce apoptosis in cells by desired method. Concurrently incubate a control culture without induction.

(2) Remove the medium and fix the cells with 50 μL 4% paraformaldehyde in PBS for 30 min at room temperature.

(3) Remove the fixation solution and wash with 200 μL PBS 3 times (5 min each time).

(4) Add 50 μL permeabilization reagent (0.3% Triton X-100 in PBS, not supplied) after the fixation, and incubate the plate for 30 min at room temperature.

(5) Wash the cells with 50 μL BSA Working Solution 3 times. (Proceed with Step B.1)

Optional: For cell slide and 24-well plate cells, the volume of fixation solution and permeabilization reagent could be adjusted according to the actual situation. You may also prepare a positive control for Tunel reaction using by digesting cells with 10 U/mL DNAase I for 10 min at room temperature before proceeding to Tunel reaction (Step B.1).

2. For non-adherent cells (Analysis by Flow Cytometry)

(1) Culture cells to an optimal density (about 1 to 2×10^6 cells/mL). Induce apoptosis by desired methods. Concurrently incubate a control culture without induction.

(2) Collect 1-5×10^6 cells by centrifugation at 300 g. Wash with 0.5 mL of PBS twice.

(3) Add 1 mL of 4% paraformaldehyde (in PBS, pH 7.4) and incubate on ice for 30 min.

(4) Centrifuge the cells at 300 g. Remove the supernatant and resuspend in 1 mL PBS. Repeat this wash twice.

(5) Resuspend cells in 500 μL 0.3% Triton-X 100 for 5 min at room temperature to permeabilize (Alternatively, resuspend the cells in 100 μg/mL Proteinase K for 5 min to permeabilize).

(6) Centrifuge the cells at 300 g. Remove the supernatant and resuspend in 1 mL PBS. Repeat this wash twice and Proceed with Step B.2.

3. For Paraffin-Embedded Tissue (Analysis by Fluorescence microscope)

(1) Deparaffinize tissue by immersing twice in xylene for 10-20 min.

(2) Rehydrate tissue by the following washes (in the order given): two washes for 5 min each in 100% ethanol, then one wash for 3 min each successively in 95%, 70%, and 50% ethanol.

(3) Wash the sample in 200-500 μL PBS twice for 5 min each.

(4) Drain excess PBS from tissue and incubate for 15 min in 20 μg/mL Proteinase K (in PBS, preparation before use) solution.

Note: The time of protease digestion will have to be optimized for specific tissue types and thicknesses. Overdigestion by protease will result in loss of cellular structure and possible release of tissue section from slide. Underdigestion will result in poor TdT labeling.

(5) Terminate the protease treatment by washing cells three times for 5 min each in PBS with gentle agitation. Proceed with Step B.1.

4. For Frozen tissue sections (Analysis by Fluorescence microscope)

(1) After sections have dried on the slide, fix with 200 μL 4% paraformaldehyde in PBS for 30min at room temperature.

(2) Wash by immersing in 200-500 μL PBS twice for 5 min each.

(3) Drain excess PBS from tissue and incubate for 15 min in 20 μg/mL Proteinase K (in PBS) solution.

(4) Terminate the protease treatment by washing cells three times for 5 min each in 200-500 μL PBS with gentle agitation. Proceed with Step B.1.

B. TUNEL assay

1. Analysis by Fluorescence microscope

(1) Prepare TdT labeling reaction buffer just before use based on the number of samples to be assayed:

Reaction Components	Volume Per Well (μL)
TdT Enzyme	1
Equilibration Buffer (5×)	10
Label Mix Green	5
deionized water	34
Total volume	50

Note: Before preparing TdT-labeled reaction buffer, rewarm each component to room temperature. The Equilibration Buffer (5×) stock solution is stored at low temperature, resulting in a small amount of component precipitation. Please invert and mix before use.

(2) Add 50 μL of the reaction mixture (from Step A.1, 3 and 4) to each sample (It is recommended 50 μL for 96-well plates, 100-200 μL for 24-well plates, Tissue sections is recommended to add 100-200 μL covering tissue) and incubate at 37℃ for 2 h (this time should be different depending on the samples) in a humidified box.

(3) Wash samples 3 times for 5 min each in PBS.

(4) Counterstain sample by incubating in 1×DAPI in PBS for 10 min.

Note: If you need to calculate the proportion of apoptotic cells, overstaining is recommended. Concentration of counterstain may have to be adjusted depending on the tissue being stained. Overstaining by DAPI may result in difficulty in observing the fluorescein label.

(5) Wash sample 3 times for 5 min each in PBS.

(6) For cell slides, paraffin sections and frozen section samples, add an aqueous mounting medium or an antifade solution, mount a coverslip and analyze using fluorescent microscopy with a fluorescein filter. For cell samples in well plates and petri dishes, add appropriate amount of PBS to immerse the cells, then take pictures and observe with a fluorescence microscope. Its signal can be easily detected at the popular FITC channel (Ex/Em = 490 nm/520 nm).

2. Analysis by Flow Cytometry

(1) Resuspend cells in 100 μL of 1×Equilibration Buffer. Incubate at room temperature for 10 min.

(2) Centrifuge cells at 300 g. Remove the supernatant and resuspend in 50 μL of TdT labeling reaction buffer. Incubate at 37℃ for 2 h (the incubation time should be different depending on the samples), during which periodically mix cells gently.

(3) Centrifuge cells at 300 g. Remove the supernatant and resuspend in 1 mL PBS. Repeat wash twice.

(4) Resuspend in 200 μL 1×DAPI in PBS. Incubate 10 min.

(5) Analyze cells by flow cytometry.

III. Detection cell apoptosis by Annexin V/PI

A. Quantification by Flow Cytometry

1. Induce apoptosis in cells using the desired method. Prepare a negative control by incubating cells in the absence of inducing agent.

2. Collect 1-2×10^5 cells by centrifugation (4℃, 300 g, 5min) and wash with ice-cold PBS twice.

Note: For adherent cells, using Trypsin (EDTA free) to digest cells firstly and then centrifugation. The time of trypsinization should not be too long, because trypsin could destroy the membrane structure.

3. Resuspend the cells in 100 μL 1×Annexin V Binding Buffer.

4. Add 4-5 μL Annexin V- AbFlour™ 488 and 1-2 μL PI to each 100 μL of cell suspension and mix gently.

5. Incubate the cells at room temperature for 15 min in the dark.

6. After the incubation period, add 400 μL 1×Annexin V Binding Buffer, mix gently, and keep the samples on ice. Analyze the cells by flow cytometry within 30 min of staining. Use 491 nm and 535 nm excitation and measure fluorescence emission near 517 nm (FITC channel) and 617 nm (PE or PI channel).

B. Detection by Fluorescence Microscopy

1. For suspension cells

(1) Follow the protocol for flow cytometry from step A.1 to step A.6.

(2) Place the cell suspension from Step A.6 on a glass slide. Cover the cells with a glass coverslip. Analyze cells by fluorescence microscopy using the appropriate filters as soon as possible (Annexin V- AbFlour™ 488 can be imaged using FITC settings, while PI can be imaged using Cy®3 or Texas Red® settings).

2. For adherent cells: the suggested protocol is as below:

(1) Grow cells on coverslips or chamber slides.

(2) Induce apoptosis in cells using the desired method. Prepare a negative control by incubating cells in the absence of inducing agent.

(3) Wash cells with PBS twice.

(4) Prepare working solution: add 4-5 μL Annexin V- AbFlour™ 488 and 1-2 μL PI to each 100 μL 1×Annexin V Binding Buffer and mix gently.

Note: The optimal concentration may need to be determined by specific experimental requirement.

(5) Add appropriate amounts of working solution to cells and incubate at room temperature for 15-30 min in the dark. (Incubation can be carried out on ice to slow down the apoptotic process, but the incubation time is extended to at least 30 min).

(6) Wash cells with 1×Annexin V Binding Buffer twice.

Note: Do not use PBS to wash cells during this step.

(7) Mount coverslips onto slides with a drop of 1×Annexin V Binding Buffer. For cells on chamber slides, add enough 1×Annexin V Binding Buffer to completely cover cells.

Note: anti-fluorescence quenching agent can also be used.

(8) Analyze cells by fluorescence microscopy using the appropriate filters as soon as possible (Annexin V- AbFlour™ 488 can be imaged using FITC settings, while PI can be imaged using Cy®3 or Texas Red® settings).

Highlight moment: In addition to detecting apoptosis based on changes in nucleus and membrane, changes in cytoplasm and mitochondria can also reflect apoptosis. Cytoplasmic changes include Caspase (KTA3020, KTA3023) and Bax (ABM0074) expression differences. Besides, decrease in mitochondrial membrane potential occurs in early apoptosis (KTA4001). Scan the QR code on the right and follow the official account of Abbkine to learn more about Abbkine products.

Typical Data

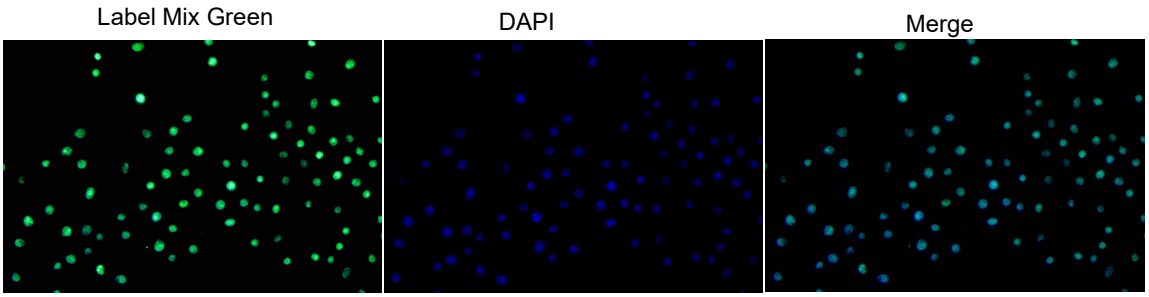

Figure 1. Hela cells were induced by this kit and were stained with TUNEL Apoptosis Detection Kit (Green Fluorescence) to detect the apoptotic effect.

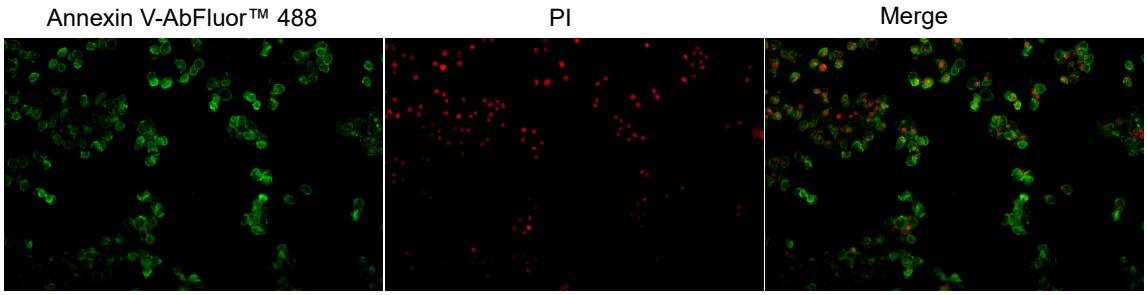

Figure 2. Hela cells were induced by this kit and were stained with Annexin V-AbFluor™ 488 Apoptosis Detection Kit to detect the apoptotic effect.

References

[1] Palanivel K, Kanimozhi V, Kadalmani B, et al. Verrucarin A induces apoptosis through ROS-mediated EGFR/MAPK/Akt signaling pathways in MDA-MB-231 breast cancer cells. J Cell Biochem, 2015; 115(11):2022-2032.

[2] Vermes I, Haanen C, Steffens-Nakken H et al. A novel assay for apoptosis.Flow cytometric detection of phosphatidylserine expression on early apoptosis cells using fluorescein labeled Annexin V. J Immunol Methods, 1995; 184:39.

[3] Gorczyca W, Gong J P, Darzynkiewicz Z.Detection of DNA strand breaks in individual apoptotic cells by the in situ terminal deoxynucleotidyl transferase and nick translation assay. Cancer Res, 1993; 53:1945.

[4] Ting Liu, Yang Li, Jiaolin Sun, et al. Engeletin suppresses lung cancer progression by inducing apoptotic cell death through modulating the XIAP signaling pathway: A molecular mechanism involving ER. Biomed Pharmacother, 2020; 128:110221.

[5] Hassan Askari, Noora Enayati, Mohammad Mehdi Ahmadian-Attari, et al. Protective Effects of Descurainia sophia against Gentamicin Induced Nephrotoxicity in Rats. Iran J Pharm Res, 2020; 20(1):40-52.

[6] Caiqin Sun, Xuesong Zhang, Fei Yu, et al. Atractylenolide I alleviates ischemia/reperfusion injury by preserving mitochondrial function and inhibiting caspase-3 activity. J Int Med Res, 2021; 49(2):300060521993315.

[7] Gu Gong, Zhifeng Han, Wenjun Wang, et al. Silencing hsa_circRNA_0008035 exerted repressive function on osteosarcoma cell growth and migration by upregulating microRNA-375. Cell Cycle, 2020; 19(17):2139-2147.

[8] Xiuli Jin, Weixin Fu, Dan Li, et al. High Expression of LINC01268 is Positively Associated with Hepatocellular Carcinoma Progression via Regulating MAP3K7. Onco Targets Ther, 2021; 14:1753-1769.

Recommended Products

Catalog No	Product Name	Recommended Reason
KTA0002	Annexin V-AbFluor™ 488 Apoptosis Detection Kit	Annexin V/PI double staining was used to detect apoptosis

KTA2010	TUNEL Apoptosis Detection Kit (Green Fluorescence)	Detection of cells and tissues apoptosis
KTA2011	TUNEL Apoptosis Detection Kit (Orange Fluorescence)	Detection of cells and tissues apoptosis
KTA4001	Mitochondrial Membrane Potential Assay Kit (JC-1)	Apoptosis was reflected by the change of membrane potential
KTA3022	Caspase-3 Assay Kit (Colorimetric)	Caspase-3 activity reflects apoptosis
KTA3026	Caspase-9 Assay Kit (Colorimetric)	Caspase-9 activity reflects apoptosis
ABM0010	Bcl-2 Monoclonal Antibody	The expression of Bcl-2 reflects apoptosis
ABM0074	Bax Mouse Monoclonal Antibody (1C1)	The expression of Bax reflects apoptosis
BMU106-EN	SuperKine™ Maximum Sensitivity Cell Counting Kit-8 (CCK-8)	Proliferation/toxicity

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.