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Technical Information

Annexin V-Flamma® Apoptosis Detection Kit

Overview

Annexin V-Flamma® apoptosis detection kit consists of Flamma® conjugated Annexin V, Propidium iodide (PI) and Aannexin V binding buffer. Phosphatidyl serine (PS) is located on the cytoplasmic surface of the cell membrane, however when apoptosis is in progress, PS is translocated to the outer leaflet of the plasma membrane exposing to the external cellular environment. Annexin V is a Ca^{2+} dependent PS-binding protein that being used to detect PS on the cell surface. PI is impermeable to live cells and the early stage apoptotic cells, but it binds to the nucleic acids of dead cells and emits red fluorescence. Normal cells are not labeled by either Annexin V or PI, early stage apoptotic cell is stained with Annexin V and natural death or cell subject to necrosis is stained with PI. The late stage apoptotic cells are stained by both reagents. This kit allows to identify apoptotic cells using fluorescence microscopic or flow cytometry analysis with simple staining procedures. Sample analysis is performed on live cells and does not require cell fixation process.

Table 1. List of Annexin V-Flamma® apoptosis detection kits

Cat. No.	Product	Ex (nm)	Em (nm)	Size
XAP1102	Annexin V-FITC Apoptosis detection kit	492	519	50 tests
XAP1102	Annexin V-FITC Apoptosis detection kit	492	519	250 tests
XAP1201	Annexin V-Flamma® 488 Apoptosis detection kit	495	519	50 tests
XAP1201	Annexin V-Flamma® 488 Apoptosis detection kit	495	519	250 tests
XAP1301	Annexin V-Flamma® 552 Apoptosis detection kit	550	565	50 tests
XAP1301	Annexin V-Flamma® 552 Apoptosis detection kit	550	565	250 tests
XAP1801	Annexin V-TAMRA Apoptosis detection kit	543	575	50 tests
XAP1801	Annexin V-TAMRA Apoptosis detection kit	543	575	250 tests

Preparation for Test

Materials required for kit but not provided

- Samples (appropriate sample concentrations range from 2×10^5 to 1×10^6 cells/mL)
- 1X Phosphate buffered saline (PBS)
- Deionized water

Handling & Storage

No.	Material	Amount*	Storage
A	Annexin V-Flamma® Flours	250 µL x 5	2 – 6 ℃, protect from light
B	Propidium iodide (PI), 1mg/ml	100 µL	2 – 6 ℃, protect from light
C	5X Annexin V binding buffer	50 mL	2 – 6 ℃, protect from light

*** for 250 tests**

- Eye contact: Immediately flush with plenty of water, remove any contact lenses and continue flushing for at least 15 minutes and see medicinal personnel.
- Skin contact: wash off immediately with soap and plenty of water while removing all contaminated clothes and shoes.
- Propidium iodide is a potential mutagen; use appropriate precautions when handling this reagent.
- Annexin V-Flamma® Flours and Propidium iodide (PI) should be stored in the dark.

Protocol

1. Induce apoptosis in cells using the desired method. Prepare a negative control by incubating cells in the absence of inducing agent.
2. After the incubation period, harvest cells, and wash the cells twice with cold PBS buffer.
3. Prepare 1X Annexin V binding buffer. For example, for making 5 mL 1X buffer, dilute 1 mL 5X Annexin V binding buffer (Component C) with 4 mL deionized water.
4. Prepare a 100 µg/mL PI working solution by diluting 5 µL of 1 mg/mL PI stock solution (Component B) with 45 µL 1X Annexin V binding buffer. Store the solution in the dark, and unused portion of PI working solution can be saved for future usage.
5. Centrifuge the washed cells from step 2, discard the supernatant and suspend the cells in 1X Annexin V binding buffer. Determine the cell density and dilute in Annexin V binding buffer to ~ 1×10^6 cells/mL.
6. Add 5 ~ 25 µL of Annexin V-Flamma® Flours (Component A) and 1 ~ 2 µL of the 100 µg/mL PI working solution (prepared in step 4) to each 100 µL of cell suspension. Higher concentrations of the Annexin V-Flamma® Flours tend to produce better results; however, the optimal staining concentration needs to be determined empirically. Incubate the cells at room temperature for 15 minutes.
7. After the incubation period, add 400 µL 1X Annexin V binding buffer, mix gently and keep the samples on ice.
8. Analyze the stained cells with flow cytometry. The population should separate into three groups: live cells show only a low level of fluorescence, apoptotic cells show corresponding Annexin V-Flamma® fluorescence, and dead cells show fluroescence of both PI and Annexin V-Flamma® Flours.

Annexin-V Flamma® Apoptosis	Fluorescence channel
Annexin V-FITC	FITC or 488
Annexin V-Flamma® 488 Apoptosis	FITC or 488
Annexin V-Flamma® 552 Apoptosis	TRITC or Cy3
Annexin V-Flamma® 648 Apoptosis	Cy5

Adherent cells with fluorescence microscopy

1. Prepare 1 x 10^5 cells/mL of cultured cells on the chamber slide. Induce apoptosis in cells using the desired method. Prepare a negative control by incubating cells in the absence of inducing agent.
2. Incubate cells in at 37 ℃ for appropriate period, After the incubation, remove supernatant, and wash the cells twice with cold PBS.
3. Prepare 1X Annexin V binding buffer. For example, to make 5 mL 1X buffer, add 1 mL 5X Annexin V binding buffer (Component C) to 4 mL deionized water.
4. Prepare a 100 µg/mL working solution of PI by diluting 5 µL of 1 mg/mL PI stock solution (Component B) in 45 µL 1X Annexin V binding buffer. Store the solution in the dark, and unused portion of this working solution saved for future experiments.
5. Re-suspend the washed cells from step 2 with 1X Annexin V binding buffer. Discard the supernatant and add 100 ~ 500 µL of 1X Annexin V binding buffer to each well.
6. Add 5 ~ 25 µL of Annexin V-Flamma® Flours (Component A) and 1 ~ 2 µL of the 100 µg/mL PI working solution (prepared in step 4) to each well. Higher concentrations of the Annexin V conjugate tend to produce better results; however, the optimal staining concentration needs to be determined empirically. Incubate the cells at room temperature for 15 minutes in the dark.
7. After the incubation period, wash the cells with 1X Annexin V binding buffer, add 500 µL 1X Annexin V binding buffer to each well and cover the well with coverslip.
8. Analyze the stained cells using fluorescence microscope. Observe the fluorescence using filters for PI and appropriate Annexin V-Flamma® fluorescence channel. The cells should separate into three groups: live, apoptotic, and dead. Live cells show only weak Annexin V staining of the cellular membrane, while apoptotic cells show a significantly higher degree of surface labeling. Dead cells show both membrane staining by Annexin V and strong nuclear staining from the propidium iodide.

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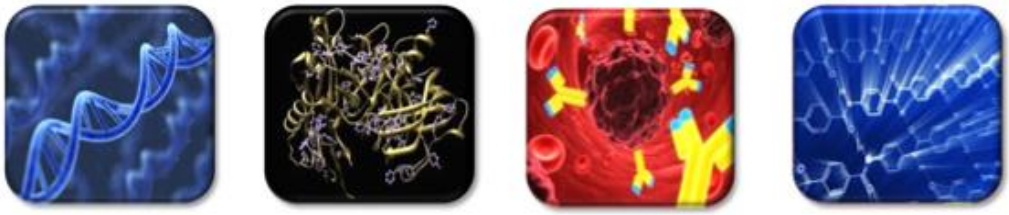
Custom Labeling Service

Based on accumulated know-how and technologies, BioActs provide a wide range of custom services such as protein fluorescence labeling, organic synthesis, oligonucleotide synthesis upon customers' request. Our reliable technology has acknowledged by our clients from domestic and overseas universities, institutions, in vitro diagnostic and pharmaceutical companies and has enabled to steadily conduct their requirements. In addition, we can introduce fluorescent materials to many other compounds such as organic and inorganic compounds, drugs, hormones, polymer, peptides, proteins, antibodies, etc. We also can provide chemical and optical analytical data, along with cell and animal experiments.



Technical Support

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SDS (Safety Data Sheets) You can find SDS at www.bioacts.com, the official website of BioActs.
CoA (Certificate of Analysis) provides detailed quality information of each product. To see CoA, check the lot number written on each product's page at www.bioacts.com, when having trouble with check, contact to our technical support team

This product can be used only for research purpose, and it can't be used to treat or diagnose human body or animals.
The product cannot be resold to other regions except for South Korea.
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