

AnnexinV-Alexa Fluor 647/7-AAD cell apoptosis assay kit



<u>Catalog Number</u>	<u>Size</u>
FXP147-020	20Tests
FXP147-050	50 Tests
FXP147-100	100 Tests

The AnnexinV-Alexa Fluor 647/7-AAD cell apoptosis assay kit can be used to detect phosphatidylserine on the outer leaflet of the cell membrane using flow cytometry. This package insert must be read in its entirety before using this product. If you have questions or experience problems with this product, please contact our Technical Support staff. Our scientists commit themselves to providing rapid and effective help.

FOR RESEARCH USE ONLY NOT FOR USE IN DIAGNOSTIC PROCEDURES

INTRODUCTION

Annexins are a family of calcium-dependent phospholipid-binding proteins. They are abundant in eukaryotic organisms belonging to a family of ubiquitous cytoplasmic proteins involved in signal transduction. All annexins have been shown to have a putative binding site for protein kinase C (PKC) but only annexin V would possess a potential pseudo- substrate site. Thus annexin V seems to modulate the activity of some PKCs on their substrates.

AnnexinV was found to play a major role in matrix vesicle-initiated cartilage calcification as a collagen-regulated calcium channel. Annexin V binds to procoagulant phospholipids (Vascular anticoagulant alpha) with high affinity.

Annexin V's preferential binding partner is phosphatidylserine (PS). PS is predominantly located in membrane leaflets, which face the cytosol. However, recent findings show that each cell type has the molecular machinery to expose PS at its cell surface. This machinery is activated during the execution of apoptosis. Once PS is exposed at the cell surface it exhibits procoagulant and proinflammatory activities. Annexin V will bind to the PS-exposing apoptotic cell and can inhibit the procoagulant and proinflammatory activities of the dying cell.

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PRINCIPLE OF THE ASSAY

Annexin V exhibits anti-phospholipase activity and binds to phosphatidylserine. FITC labelling allows simple direct detection by FACS analysis. Counterstaining by 7-AAD allows the discrimination of apoptotic cells.

REAGENTS

Table 1: Reagents of the Kit

	Catalog Number FXP147		
	20 tests	50 tests	100 tests
rh AnnexinV-Alexa Fluor 647	1vial (0.1ml)	1vial (0.25ml)	1vial (0.5ml)
Binding Buffer (4x)	1 bottle (4 ml)	1 bottle (10 ml)	1bottle (20ml)
7-AAD (20 µg/ml)	1vial (0.2ml)	1vial (0.5ml)	1vial (1.0ml)

STORAGE

Store kit reagents between 2° and 8°. Immediately after use remaining reagents should be returned to cold storage (2° to 8°C). Expiry of the kit and reagents is stated on labels.

Expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, this reagent is not contaminated by the first handling.

THE REQUIRED ITEMS (not provided, but can help to buy):

1. 5 ml and 10 ml graduated pipettes
2. 5 µl to 1000 µl adjustable single channel micropipettes with disposable tips
3. Beakers, flasks, cylinders necessary for preparation of reagents
4. Glass-distilled or deionized water
5. Bench top centrifuge
6. Flow Cytometer
7. PBS (for 1 liter: 8.00g NaCl, 0.20 g KCl, Na₂HPO₄ • 12 H₂O 2.85 g, 0.20 g KH₂PO₄)

PRECAUTIONS FOR USE

1. All reagents should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash

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immediately with water.

2. Do not mix or substitute reagents with those from other lots or other sources.
3. Do not use kit reagents beyond expiration date on label.
4. Do not expose kit reagents to strong light during storage or incubation.
5. Do not eat or smoke in areas where kit reagents or samples are handled.
6. Avoid contact of skin or mucous membranes with kit reagents or specimens.
7. Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.
8. Avoid splashing or generation of aerosols.
9. In order to avoid microbial contamination or cross-contamination of reagents which may invalidate the test use disposable pipette tips and/or pipettes.
10. Glass-distilled water or deionized water must be used for reagent preparation.
11. Decontaminate and dispose specimens and all potentially contaminated materials as they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C.
12. Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0% sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.

REAGENT PREPARATION

Dilute Binding Buffer (4x) 1:4 in distilled water (1 ml binding buffer and 3 ml distilled water).

GENERAL PROTOCOL

1. Wash cells in PBS(4°C) by gentle shaking or pipetting up and down.
2. Resuspend cells in Binding Buffer (1x); cell density should be $1-2 \times 10^6$ /ml.
3. Take 100 μ l resuspend cells to the bottom of 5ml tube; Add 5 μ l AnnexinV-Alexa Fluor 647 and 10 μ l 7-AAD (20 μ g/ml) to 100 μ l cell suspension.
4. Mix and incubate for 15 min at room temperature away from light.
5. Add 400 μ l PBS to tube. Perform FACS analysis.

Note: cannot be used trypsin with EDTA when digestive cells.

Limitations

Bacterial or fungal contamination of either screen samples or reagents or cross-contamination

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between reagents may cause erroneous results.

Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergent before use.

RELATED PRODUCTS

Table 2: Related products

Catalog number	Products name	size
Annexin V-FITC/PI cell apoptosis assay kit	FXP018	20T/50T/100T
Annexin V-eGFP cell apoptosis assay kit	FXP025	20T/50T/100T
AnnexinV Alexa Fluor 488/PI cell apoptosis assay kit	FXP022	20T/50T/100T
AnnexinV Alexa Fluor 647/PI cell apoptosis assay kit	FXP023	20T/50T/100T
Annexin V PE/7-AAD cell apoptosis assay kit	FXP027	20T/50T/100T
AnnexinV-FITC/7-AAD cell apoptosis assay kit	FXP145	20T/50T/100T
AnnexinV-Alexa Fluor 488/7-AAD cell apoptosis assay kit	FXP147	20T/50T/100T
AnnexinV-Alexa Fluor 647/7-AAD cell apoptosis assay kit	FXP147	20T/50T/100T

If you have any questions, please tell us!

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