



## **Annexin V-Cy3 Apoptosis Detection Kit**

*Manufactured by BioVision.*

**ALX-850-252-KI01: ~25 tests**

**ALX-850-252-KI02: ~100 tests**

**ALX-850-252-KI03: ~400 tests**

(Version 01: December 1, 2004)

### **NORTH AMERICA**

AXXORA, LLC  
6181 Cornerstone Court East  
Suite 103  
San Diego, CA 92121-4727  
Phone: (858) 550-8828  
Fax: (858) 550-8825  
E-mail: [axxora-usa@axxora.com](mailto:axxora-usa@axxora.com)

### **SWITZERLAND/REST OF THE WORLD**

ALEXIS CORPORATION  
Industriestrasse 17, Postfach  
CH-4415 Lausen / Switzerland  
Phone: +41 61 926 89 89  
Fax: +41 61 926 89 79  
E-mail: [alexis-ch@alexis-corp.com](mailto:alexis-ch@alexis-corp.com)

### **GERMANY**

AXXORA DEUTSCHLAND GmbH  
Marie-Curie-Strasse 8  
DE-79539 Lörrach  
Phone: (07621) 5500 522  
Fax: (07621) 5500 523  
E-mail: [axxora-de@axxora.com](mailto:axxora-de@axxora.com)

### **UK & IRELAND**

AXXORA (UK) LTD.  
P.O. Box 6757  
Bingham, Nottingham NG13 8LS  
Phone: +44 1949 836111  
Fax: +44 1949 836222  
E-mail: [axxora-uk@axxora.com](mailto:axxora-uk@axxora.com)

**For laboratory use only. Not for human or diagnostic use.**

## I. Introduction:

The **Annexin V-Cy3 Apoptosis Detection Kit** is based on the observation that soon after initiating apoptosis, most cell types translocate the membrane phosphatidylserine (PS) from the inner face of the plasma membrane to the cell surface. Once on the cell surface, PS can be easily detected by staining with a fluorescent conjugate of Annexin V, a protein that has a high affinity for PS (1,2). The one-step staining procedure takes only 10 minutes. Detection can be analyzed by flow cytometry or by fluorescence microscopy.

## II. Kit Contents:

Components	850-252-KI01	850-252-KI02	850-252-KI03
	25 tests	100 tests	400 tests
Annexin V-Cy3	125 µl	500 µl	2 ml
1x Binding Buffer	12.5 ml	50 ml	2 x 100 ml

## II. Annexin V-Cy3 Assay Protocol:

### A. Incubation of cells with Annexin V-Cy3

1. Induce apoptosis by desired method.
2. Collect  $1-5 \times 10^5$  cells by centrifugation.
3. Resuspend cells in 500 µl of 1x Binding Buffer.
4. Add 5 µl of Annexin V-Cy3.
5. Incubate at room temperature for 5 minutes in the dark.  
Proceed to B or C below depending on method of analysis.

### B. Quantification by Flow Cytometry

Analyze Annexin V-Cy3 binding by flow cytometry (Ex = 543 nm; Em = 570 nm) using the phycoerythrin emission signal detector (usually FL2).

For analyzing adherent cells, gently trypsinize and wash cells once with serum-containing media before incubation with Annexin V-Cy3 (A.3-5).

### C. Detection by Fluorescence Microscopy

1. Place the cell suspension from Step A.5 on a glass slide. Cover the cells with a glass coverslip.

For analyzing adherent cells, grow cells directly on a coverslip. Following incubation (A.5), invert coverslip on glass slide and visualize cells. The cells can also be washed and fixed in 2% formaldehyde before visualization. (Cells must be incubated with Annexin V-Cy3 before fixation since any cell membrane disruption can cause nonspecific binding of Annexin V to PS on the inner surface of the cell membrane.)

2. Observe the cells under a fluorescence microscope using a rhodamine filter.

Cells which have bound Annexin V-Cy3 will show red staining in the plasma membrane.

### III. Storage and stability:

Store kit at 4°C. PROTECT FROM LIGHT!

### IV. References:

*Annexin V for flow cytometric detection of phosphatidylserine expression on B cells undergoing apoptosis:* G. Koopman, et al.; *Blood* **84**, 1415 (1994)

*Early redistribution of plasma membrane phosphatidylserine is a general feature of apoptosis regardless of the initiating stimulus: inhibition by overexpression of Bcl-2 and Abl:* S.J. Martin, et al.; *J. Exp. Med.* **182**, 1545 (1995)