Technical Data Sheet

# Cy™5 Annexin V

## Product Information

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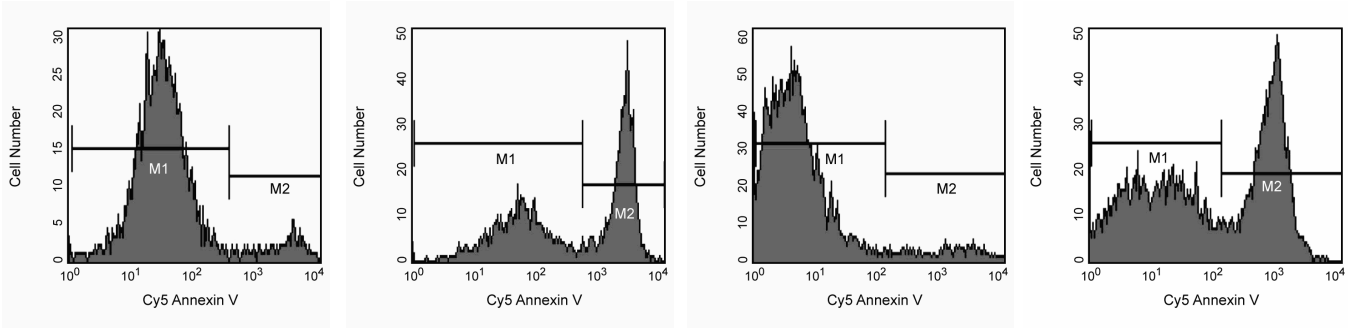
|---|---|
| **Material Number:** | **559934** |
| **Size:** | 200 tests |
| **Vol. per Test:** | 5 µl |
| **Storage Buffer:** | Aqueous buffered solution containing BSA and ≤0.09% sodium azide. |

## Description

Apoptosis is a normal physiologic process which occurs during embryonic development as well as in maintenence of tissue homeostasis. The apoptotic program is characterized by certain morphologic features, including loss of plasma membrane asymmetry and attachment, condensation of the cytoplasm and nucleus, and internucleosomal cleavage of DNA. Loss of plasma membrane is one of the earliest features. In apoptotic cells, the membrane phospholipid phosphatidylserine (PS) is translocated from the inner to the outer leaflet of the plasma membrane, thereby exposing PS to the external cellular environment. Annexin V is a 35-36 kDa Ca2+ dependent phospholipid-binding protein that has a high affinity for PS, and binds to cells with exposed PS. Annexin V may be conjugated to fluorochromes including Cy5. This format retains its high affinity for PS and thus serves as a sensitive probe for flow cytometric analysis of cells that are undergoing apoptosis. Since externalization of PS occurs in the earlier stages of apoptosis, Cy5 Annexin V staining can identify apoptosis at an earlier stage than assays based on nuclear changes such as DNA fragmentation.

Cy5 Annexin V staining precedes the loss of membrane integrity which accompanies the latest stages of cell death resulting from either apoptotic or necrotic processes. Therefore, staining with Cy5 Annexin V is typically used in conjunction with a vital dye such as propidium iodide (PI) or 7-Amino-Actinomycin (7-AAD) to allow the investigator to identify early apoptotic cells (7-AAD negative, Cy5 Annexin V positive). Viable cells with intact membranes exclude 7-AAD, whereas the membranes of dead and damaged cells are permeable to 7-AAD. For example, cells that are considered viable are both Cy5 Annexin V and 7-AAD negative while cells that are in early apoptosis are Cy5 Annexin V positive and 7-AAD negative, while cells that are in late apoptosis or already dead are both Cy5 Annexin V and 7-AAD positive. This assay does not distinguish between cells that have undergone apoptotic death versus those that have died as a result of a necrotic pathway because in either case, the dead cells will stain with both Cy5 Annexin V and 7-AAD. However, when apoptosis is measured over time, cells can be often tracked from Cy5 Annexin V and 7-AAD negative (viable, or no measurable apoptosis), to Cy5 Annexin V positive and 7-AAD negative (early apoptosis, membrane integrity is present) and finally to Cy5 Annexin V and 7-AAD positive (end stage apoptosis and death). The movement of cells through these three stages suggests apoptosis. In contrast, a single observation indicating that cells are both Cy5 Annexin V and 7-AAD positive, in of itself, reveals less information about the process by which the cells underwent their demise.




***Cy5 Annexin V: A tool for identifying cells that are undergoing apoptosis.*** *Jurkat T cells were left untreated (far left & center right panels) or treated for 6 hours (center left & far right panels) with 6 µM camptothecin. Cells were incubated with Cy5 Annexin V and analyzed by flow cytometry. Left panels are representative of an exerpiment analyzed on a BD FACSCalibur™ instrument and right panels from an experiment analyzed on a BD FACSVantage ™. Untreated cells were primarily Cy5 Annexin V negative, indicating that they were viable and not undergoing apoptosis. After a 4 hour treatment with camptothecin, there were two populations of cells: cells undergoing apoptosis (Cy5 Annexin V positive), and cells that were viable and not undergoing apoptosis (Cy5 Annexin V negative).*

## Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

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## Application Notes

**Application**

| | |
|---|---|
| Flow cytometry | Routinely Tested |

**Recommended Assay Procedure:**

Cy5 Annexin V is a sensitive probe for identifying apoptotic cells, binding to negatively charged phospholipid surfaces (Kd of ~5 x 10e2) with a higher affinity for phosphatidylserine (PS) than most other phospholipids. Cy5 Annexin V binding is calcium dependent and defined calcium and salt concentrations are required for optimal staining as described in the Cy5 Annexin V Staining Protocol. **Investigators should note that Cy5 Annexin V flow cytometric analysis on adherent cell types (e.g HeLa, NIH 3T3, etc.) is not routinely tested as specific membrane damage may occur during cell detachment or harvesting. Methods for utilizing Annexin V for flow cytometry on adherent cell types, however, have been previously reported (Casiola-Rosen et al. and van Engelend et al.).**

**INDUCTION OF APOPTOSIS BY CAMPTOTHECIN**

The following protocol is provided as an illustration on how Cy5 Annexin V may be used on a cell line (Jurkat).

**Materials**

1. Prepare Camptothecin stock solution (Sigma-Aldrich Cat. No. C-9911): 1 mM in DMSO.
2. Jurkat T cells (ATCC TIB-152).

**Procedure**

1. Add Camptothecin (final conc. 4-6 µM) to 1 x 10e6 Jurkat cells.
2. Incubate the cells for 4-6 hr at 37°C.
3. Proceed with the Cy5 Annexin V Staining Protocol to measure apoptosis.

**Cy5 ANNEXIN V STAINING PROTOCOL**

**Reagents**

1. Cy5 Annexin V: Included. Use 5 µl per test.
2. 7-Amino-Actinomycin D (7-AAD): Not included. 7-AAD (Cat.No. 559925) is a convenient, ready-to-use nucleic acid dye with fluorescence detectable in the far red range of the spectrum. Use 5 µl per test.
3. 10X Binding Buffer: Not Included. 0.1 M Hepes (pH 7.4) 1.4 M NaCl, 25 mM CaCl2. Store at 4°C. Alternatively, catalog number 556454 may be purchased.

**Staining**

1. Wash cells twice with cold PBS and then resuspend cells in 1X Binding Buffer at a concentration of 1 x 10e6 cells/ml.
2. Transfer 100 µl of the solution (1 x 10e5 cells) to a 5 ml culture tube.
3. Add 5 µl of Cy5 Annexin V (for one and two color analysis) and 5 µl of 7-AAD (for two color analysis only).
4. Gently vortex the cells and incubate for 15 min at RT (25°C) in the dark.
5. Add 400 µl of 1X Binding Buffer to each tube. Analyze by flow cytometry within 1 hr.

**SUGGESTED CONTROLS FOR SETTING UP FLOW CYTOMETRY**

Cy5 emission wavelength is 670 nm and excitation is 625-650 nm. Cy5 is optimized for FL4 fluorescence on the BD FACSCalibur™ flow cytometer. For the BD FACSVantage™ flow cytometer, the recommended filter for emission is 675/20.

**The following controls are used to set up compensation and quadrants:**

1. Unstained cells.
2. Cells stained with Cy5 Annexin V alone (no 7-AAD).
3. Cells stained with 7-AAD alone (no Cy5 Annexin V).

**Other Staining Controls**

A cell line that can be easily induced to undergo apoptosis should be used to obtain positive control staining with Cy5 Annexin V and/or Cy5 Annexin V and 7-AAD. It is important to note that the basal level of apoptosis and necrosis varies considerably within a population. Thus, even in the absence of induced apoptosis, most cell populations will contain a minor percentage of cells that are positive for apoptosis (Cy5 Annexin V positive, 7-AAD negative or Cy5 Annexin V positive, 7-AAD positive).

The untreated population is used to define the basal level of apoptotic and dead cells. The percentage of cells that have been induced to undergo apoptosis is then determined by subtracting the percentage of apoptotic cells in the untreated population from percentage of apoptotic cells in the treated population. Since cell death is the eventual outcome of cells undergoing apoptosis, cells in the late stages of apoptosis will have a damaged membrane and stain positive for 7-AAD as well as for Cy5 Annexin V. Thus, the assay does not distinguish between cells that have already

undergone an apoptotic cell death and those that have died as a result of necrotic pathway, because in either case the dead cells will stain with both Cy5 Annexin V and 7-AAD.

## Suggested Companion Products

| Catalog Number | Name | Size | Clone |
|---|---|---|---|
| 559925 | 7-AAD | 2.0 ml | (none) |
| 556454 | Annexin V Binding Buffer, 10X concentrate | 50 ml | (none) |

## Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
4. This product is subject to proprietary rights of Amersham Biosciences Corp. and Carnegie Mellon University and made and sold under license from Amersham Biosciences Corp. This product is licensed for sale only for research. It is not licensed for any other use. If you require a commercial license to use this product and do not have one return this material, unopened to BD Biosciences, 10975 Torreyana Rd, San Diego, CA 92121 and any money paid for the material will be refunded.
5. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
6. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

## References


Homburg CH, de Haas M, von dem Borne AE, Verhoeven AJ, Reutelingsperger CP, Roos D. Human neutrophils lose their surface Fc gamma RIII and acquire Annexin V binding sites during apoptosis in vitro. *Blood.* 1995; 85(2):532-540.(Biology: Apoptosis)

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Martin SJ, Reutelingsperger CP, McGahon AJ, et al. 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. *J Exp Med.* 1995; 182(5):1545-1556.(Biology: Apoptosis)

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Vermes I, Haanen C, Steffens-Nakken H, Reutelingsperger C. A novel assay for apoptosis. Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled Annexin V. *J Immunol Methods.* 1995; 184(1):39-51.(Methodology: Apoptosis, Flow cytometry)