Technical Data Sheet

V500 Annexin V

Product Information

Material Number:	**561501**
Size:	50 tests
Vol. per Test:	5 µl
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

Apoptosis is a normal physiologic process that occurs during embryonic development as well as in maintenance 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 BD Horizon™ V500. 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, V500 Annexin V staining can identify apoptosis at an earlier stage than assays based on nuclear changes such as DNA fragmentation.

V500 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 V500 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 (PI negative, V500 Annexin V positive). Viable cells with intact membranes exclude PI, whereas the membranes of dead and damaged cells are permeable to PI. For example, cells that are considered viable are both V500 Annexin V and PI negative while cells that are in early apoptosis are V500 Annexin V positive and PI negative, while cells that are in late apoptosis or already dead are both V500 Annexin V and PI 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 V500 Annexin V and PI. However, when apoptosis is measured over time, cells can be often tracked from V500 Annexin V and PI negative (viable, or no measurable apoptosis), to V500 Annexin V positive and PI negative (early apoptosis, membrane integrity is present) and finally to V500 Annexin V and PI 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 V500 Annexin V and PI positive, in of itself, reveals less information about the process by which the cells underwent their demise.

The Annexin V is conjugated to BD Horizon™ V500 that has been developed for use in multicolor flow cytometry experiments and is available exclusively from BD Biosciences. It is excited by the Violet laser with an Ex max of 415 nm and Em Max at 500 nm. BD Horizon V500 conjugates emit at a similar wavelength to Amcyan yet exhibit reduced spillover into the FITC channel. For more information on BD Horizon V500, visit bdbiosciences.com/colors.

When compensating dyes in this spectral range (such as Horizon™ V500 and AmCyan), the most accurate compensation can be obtained using single stained cellular controls. Due to spectral differences between cells and beads in this channel, using BD CompBeads can result in spillover errors for V500 and AmCyan reagents. Therefore, the use of BD CompBeads or BD CompBeads Plus to determine spillover values for these reagents is not recommended. Different V500 reagents (e.g. CD4 vs. CD45) can have slightly different fluorescence spillover therefore, it may also be necessary to use clone specific compensation controls when using these reagents.

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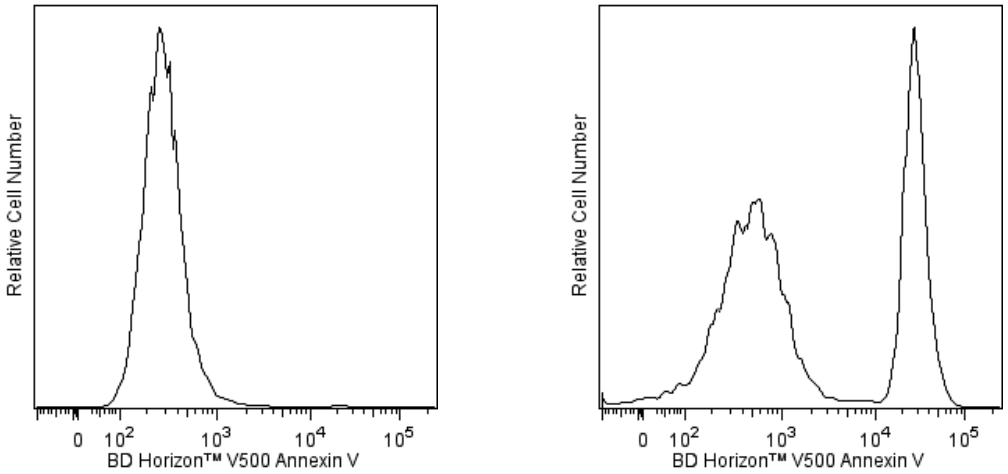

Staining cells with BD Horizon™ V500 Annexin V and multicolor flow cytometric analysis of cells undergoing apoptosis. *Jurkat T cells (Human T-cell leukemia) were left untreated (Left Panel) or treated for 4 hours (Right Panel) with 6 µM camptothecin. Cells were incubated with BD Horizon™ V500 Annexin V (Cat. No. 561501) and analyzed by flow cytometry. Untreated cells were primarily V500 Annexin V negative, indicating that they were viable and not undergoing apoptosis (Left Panel). After a 4 hour treatment with camptothecin, there were two populations of cells: cells undergoing apoptosis (V500 Annexin V positive), and cells that were viable and not undergoing apoptosis (V500 Annexin V negative) (Right Panel). Flow cytometry was performed using a BD™ LSR II Flow Cytometer System.*

Preparation and Storage

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

Application Notes

Application

Flow cytometry	Routinely Tested

Recommended Assay Procedure:

BD™ Horizon V500 Annexin V is a sensitive probe for identifying apoptotic cells, binding to negatively charged phospholipid surfaces with a higher affinity for phosphatidylserine (PS) than most other phospholipids. V500 Annexin V binding is calcium dependent and defined calcium and salt concentrations are required for optimal staining as described in the V500 Annexin V Staining Protocol. **Investigators should note that V500 Annexin V flow cytometric analysis on adherent cell types (eg, 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 V500 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 × 10^6 Jurkat cells.
2. Incubate the cells for 4-6 hr at 37°C.
3. Proceed with the V500 Annexin V Staining Protocol to measure apoptosis.

V500 ANNEXIN V STAINING PROTOCOL

Reagents

1. V500 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 Annexin Binding Buffer: Not Included. 0.1 M Hepes (pH 7.4) 1.4 M NaCl, 25 mM CaCl2. Store at 4°C. Alternatively, BD Pharmingen™ Annexin V Binding Buffer, 10X concentrate (Cat. No. 556454) may be purchased.

Staining

1. Wash cells twice with cold PBS and then resuspend cells in 1× Binding Buffer at a concentration of 1 × 10^6 cells/ml.
2. Transfer 100 µl of the solution (1 × 10^5 cells) to a 5 ml culture tube.
3. Add 5 µl of V500 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 1× Binding Buffer to each tube. Analyze by flow cytometry within 1 hr.

SUGGESTED CONTROLS FOR SETTING UP FLOW CYTOMETRY

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

1. Unstained cells.
2. Cells stained with V500 Annexin V alone (no 7-AAD).
3. Cells stained with 7-AAD alone (no V500 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 V500 Annexin V and/or V500 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 (V500 Annexin V positive, 7-AAD negative or V500 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 the 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 V500 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 V500 Annexin V and 7-AAD.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 ml	(none)
556454	Annexin V Binding Buffer, 10X concentrate	50 ml	(none)
559925	7-AAD	2.0 ml	(none)

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10^6 cells in a 100-µl experimental sample (a test).
2. BD Horizon™ V500 has a maximum absorption of 415 nm and maximum emission of 500 nm. Before staining with this reagent, please confirm that your flow cytometer is capable of exciting the fluorochrome and discriminating the resulting fluorescence.
3. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
4. 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.
5. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
6. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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