Technical Data Sheet

PerCP-Cy™5.5 Annexin V

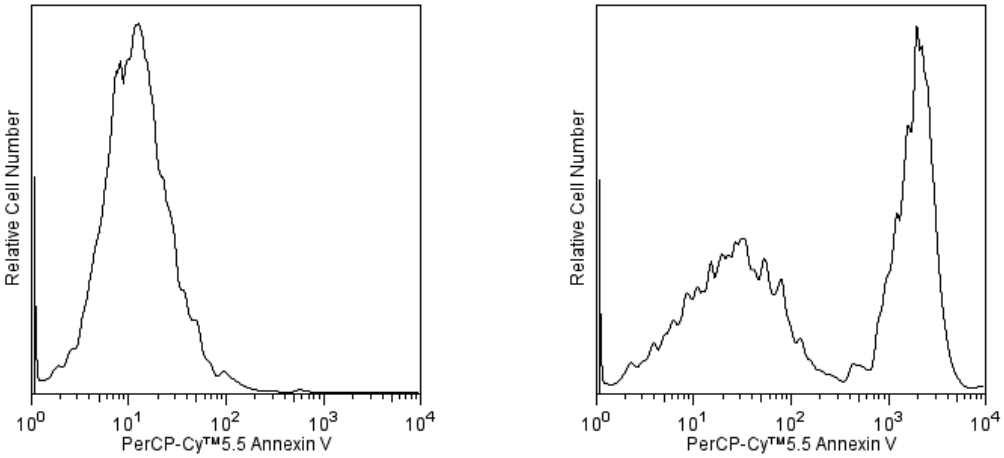
Product Information

Material Number:	**561431**
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 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 PerCP-Cy™5.5 Annexin V. 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, PerCP-Cy™5.5 Annexin V staining can identify apoptosis at an earlier stage than assays based on nuclear changes such as DNA fragmentation.

NOTE: Investigators should note that the use of Propidium Iodide (PI) or 7-Amino-Actinomycin D (7-AAD) for co-staining with PerCP-Cy5.5 Annexin V is not recommended due to spectral overlap.



Staining cells with PerCP-Cy™5.5 Annexin V and multicolor flow cytometric analysis of cells undergoing apoptosis. *Jurkat T cells were left untreated (Left Panel) or treated for 4 hours (Right Panel) with 6 µM camptothecin. Cells were incubated with PerCP-Cy™5.5 Annexin V (Cat. No. 561431) and analyzed by flow cytometry. Untreated cells were primarily PerCP-Cy™5.5 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 (PerCP-Cy™5.5 Annexin V positive), and cells that were viable and not undergoing apoptosis (PerCP-Cy™5.5 Annexin V negative) (Right Panel). Flow cytometry was performed using a BD™ LSR II Flow Cytometer System.*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated with PerCP-Cy5.5 under optimum conditions, and unconjugated antibody and free PerCP-Cy5.5 were removed. Storage of PerCP-Cy5.5 conjugates in unoptimized diluent is not recommended and may result in loss of signal intensity. 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:

PerCP-Cy™5.5 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. PerCP-Cy™5.5 Annexin V binding is calcium dependent and defined calcium

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and salt concentrations are required for optimal staining as described in the PerCP-Cy™5.5 Annexin V Staining Protocol. **Investigators should note that PerCP-Cy™5.5 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 PerCP-Cy™5.5 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 PerCP-Cy™5.5 Annexin V Staining Protocol to measure apoptosis.

PerCP-Cy™5.5 ANNEXIN V STAINING PROTOCOL

Reagents

1. PerCP-Cy™5.5 Annexin V: Included. Use 5 µl per test.
2. 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 PerCP-Cy™5.5 Annexin V.
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 markers for quantifying PerCP-Cy™5.5 Annexin V-positive cells:

1. Unstained cells.
2. Cells stained with PerCP-Cy™5.5 Annexin V alone.

Other Staining Controls

A cell line that can be easily induced to undergo apoptosis should be used to obtain positive control staining with PerCP-Cy™5.5 Annexin V. 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 (PerCP-Cy™5.5 Annexin V positive cell).

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.

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. Please observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem fluorochrome conjugate; therefore, we recommend that special precautions be taken (such as wrapping vials, tubes, or racks in aluminum foil) to prevent exposure of conjugated reagents, including cells stained with those reagents, to room illumination.
2. PerCP-Cy5.5 is optimized for use with a single argon ion laser emitting 488-nm light. Because of the broad absorption spectrum of the tandem fluorochrome, extra care must be taken when using dual-laser cytometers, which may directly excite both PerCP and Cy5.5™. We recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.

3. PerCP-Cy5.5–labelled antibodies can be used with FITC- and R-PE–labelled reagents in single-laser flow cytometers with no significant spectral overlap of PerCP-Cy5.5, FITC, and R-PE fluorescence.
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. Cy is a trademark of Amersham Biosciences Limited. This conjugated product is sold under license to the following patents: US Patent Nos. 5,486,616; 5,569,587; 5,569,766; 5,627,027.
6. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
7. 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.
8. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
9. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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