

## Technical Data Sheet

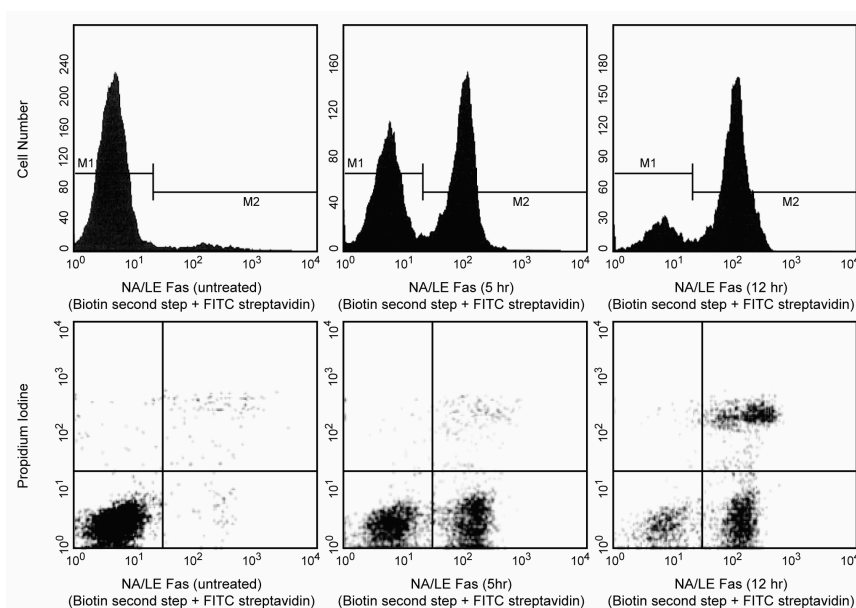
## Propidium Iodide Staining Solution

## Product Information

**Material Number:** 556463  
**Size:** 2.0 ml  
**Storage Buffer:** Aqueous buffered solution containing no preservative.

## Description

The Propidium Iodide (PI) Staining Solution may be used to assess plasma membrane (PM) integrity in Annexin V apoptosis assays. PI is a fluorescent vital dye that stains DNA. It does not cross the PM of cells that are viable or in the early stages of apoptosis because they maintain PM integrity. In contrast those cells in the late stages of apoptosis or already dead have lost PM integrity and are permeable to PI. PI is detected in the orange range of the spectrum using a 562-588 nm band pass filter. Annexin V binds to cells early in apoptosis, and continues to be bound through cell death. PI is used in two-color Annexin V flow cytometric assays to distinguish cells that are in the earlier stages of apoptosis (Annexin V positive, PI negative) from those that are in the later stages of apoptosis or already dead (Annexin V positive, PI positive).



**Flow cytometric analysis of cells following induction of apoptosis.** Jurkat leukemia cells were left untreated (left top & left bottom panels) or treated for 5 hours (middle top & middle bottom panels) or 12 hours (right top & right bottom panels) with anti-human Fas antibody (clone DX2, Cat. No. 555670) and Protein G (the addition of Protein G enhances the ability of DX2 to induce apoptosis, presumably by cross-linking Fas). Cells were incubated with Annexin V-Biotin, (Cat. No. 556417) followed by incubation with SAV-FITC (Cat. No. 554060) in Propidium Iodide (PI) Staining Solution (Cat. No. 556463). Cells were then analyzed by flow cytometry. Untreated cells were primarily Annexin V-Biotin and PI negative, indicating that they were viable and not undergoing apoptosis. After a 5 hour treatment with DX2, there were two populations of cells: Cells undergoing apoptosis (Annexin V-Biotin positive and PI negative), and cells that were viable and not undergoing apoptosis (Annexin V-Biotin and PI negative). After a 12 hour treatment with DX2, three populations of cells were identified: Cells that had already died or were in late stage of apoptosis (Annexin V-Biotin and PI positive), cells undergoing apoptosis (Annexin V-Biotin positive and PI negative), and cells that were viable and not undergoing apoptosis (Annexin V-Biotin and PI negative).

## Preparation and Storage

Store undiluted at 4° C and protected from prolonged exposure to light. Do not freeze.  
 This preparation contains no preservatives, thus it should be handled under aseptic conditions.  
 The PI Staining Solution is composed of 50 µg PI/ml in PBS (pH 7.4) and is 0.2 µm sterile filtered.

## Application Notes

## Application

Flow cytometry

Routinely Tested

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**Recommended Assay Procedure:**

The PI Staining Solution is designed for use in two-color Annexin V flow cytometric assays. It can be used in conjunction with Annexin V conjugated to fluorescein isothiocyanate (Annexin V-FITC, Cat. No. 556419) or biotinylated Annexin V (Annexin V-Biotin, Cat. No. 556417). Suggested amounts to use are 10 µl/test (1x10<sup>6</sup> cells).

**Caution:** Propidium Iodide is a potential carcinogen. Avoid contact with skin and eyes. Wear suitable protective clothing, gloves, and eye/face protection.

**Suggested Companion Products**

<b>Catalog Number</b>	<b>Name</b>	<b>Size</b>	<b>Clone</b>
556419	FITC Annexin V	200 tests	(none)
556417	Annexin V-Biotin	200 tests	(none)
554060	FITC Streptavidin	0.5 mg	(none)
555670	Purified NA/LE Mouse Anti-Human CD95	0.5 mg	DX2

**Product Notices**

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 X 10<sup>6</sup> cells in a 100-µl experimental sample (a test).
3. Please refer to [www.bdbiosciences.com/pharming/en/protocols](http://www.bdbiosciences.com/pharming/en/protocols) for technical protocols.

**References**

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.(Methodology: Apoptosis, Flow cytometry)