Violet Ratiometric Membrane Asymmetry Probe/Dead Cell Apoptosis Kit

Catalog no. A35137

Table 1. Contents and storage information.

Material	Amount	Storage	Stability
F2N12S (Component A)	100 µL	• ≤–20°C • Dessicate • Protect from light	When stored as directed, this kit is stable for at least 1 year.
SYTOX® AADvanced™ dead cell stain (Component B)	1 vial		
Dimethylsulfoxide (DMSO), anhydrous (Component C)	200 µL	≤–20°C	
Number of assays: Sufficient material is supplied for 100 reactions, based on the protocol below.			
Approximate fluorescence excitation/emission maxima: F2N12S**:** 405/530 nm for apoptotic and dead cells, and 405/585 nm for live cells; SYTOX® AADvanced™ dead cell stain: 546/647 nm, bound to DNA.			

Introduction

Apoptosis is a carefully regulated and essential part of normal tissue development and homeostasis. Regulatory changes in the apoptotic pathway have been implicated in many diseases, most notably many types of cancers and autoimmune disorders. Normal cells exhibit a remarkable asymmetry in lipid distribution between the outer and inner cell membranes characterized by phosphatidyl-serine (PS) and phosphatidylethanolamine (PE), normally located on the inner leaflet of the cell membrane. During apoptosis PS and PE are translocated from the inner to outer leaflet of the cell membrane.[1] Translocation of PE and PS to the external cellular environment facilitates the recognition and elimination of these cells by macrophages.[2, 3]

The Violet Ratiometric Membrane Asymmetry Probe, 4'-N,N-diethylamino-6-(N,N,N-dodecyl-methylamino-sulfopropyl)-methyl-3-hydroxyflavone (**F2N12S**), is a novel violet excitable dye for the detection of membrane asymmetry changes during apoptosis. The dye exhibits an excited-state intramolecular proton transfer (ESIPT) reaction resulting in a dual fluorescence with two emission bands corresponding to 530 nm and 585 nm, producing a two-color ratiometric response to variations in surface charge.[4] Ratiometric probes have several advantages over traditional fluorochrome labeled reagents. The ratiometric probe is a self-calibrating absolute parameter of apoptotic transformation, which is independent of probe concentration, cell size, and instrument variation, such as fluctuations of laser intensity or sensitivity of the detectors.[4] Given that apoptosis modifies the surface charge of the outer leaflet of the plasma membrane, the violet membrane asymmetry probe F2N12S can monitor changes in membrane asymmetry that occur during apoptosis through a change in the relative intensity of the two emission bands of the dye (Figure 1).

The Violet Ratiometric Membrane Asymmetry Probe/Dead Cell Apoptosis Kit provides a simple and fast method for the detection of apoptosis with dead cell discrimination by flow

cytometry. Samples can be analyzed after a 5 minute incubation at room temperature, and the assay does not require special buffers or wash steps. This kit can be paired with other reagents such as MitoProbe™ $DiIC_1(5)$ or annexin V for multiparametric analysis of apoptosis and viability.

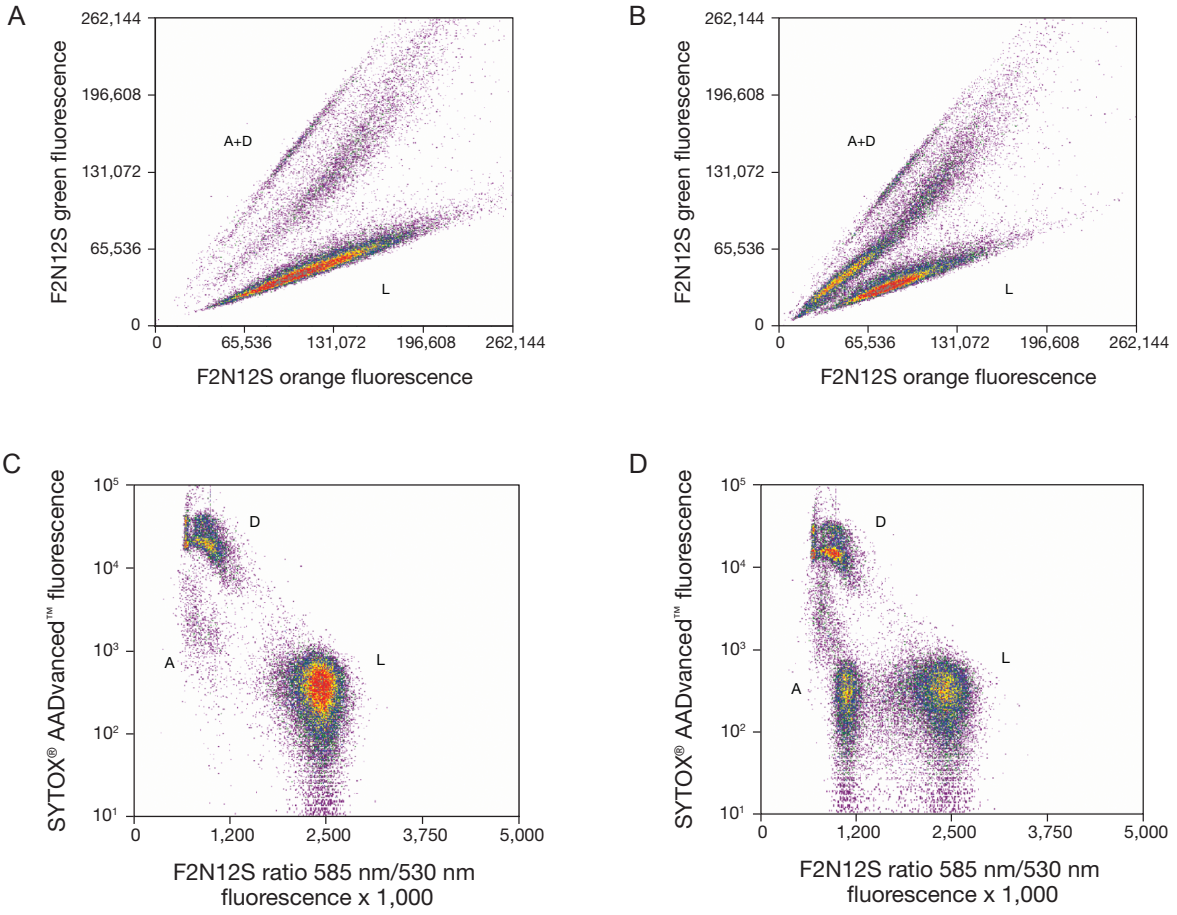

Figure 1. Jurkat cells (T-cell leukemia, human) treated with 10 µM camptothecin for four hours (panels B and D) or untreated control (panels A and C). Cells were stained according to the protocol. Samples were analyzed on a flow cytometer with 405 nm excitation using 585 nm and 530 nm bandpass filters for F2N12S and 488 nm excitation for SYTOX® AADvanced™ dead cell stain using a 695 nm bandpass filter. In panels A and B, living cells can be discriminated from apoptotic and dead cells by the relative intensities of the two emission bands from F2N12S. In panels C and D, SYTOX® AADvanced™ dead cell stain fluorescence is plotted against a derived ratio parameter from the two emission bands (585/530 nm) of F2N12S. A = apoptotic cells, L = live cells, D = dead cells.

Before Starting

Materials Required but Not Provided

- Cells of interest as a single-cell suspension
- HBSS or similar buffer without protein
- Flow cytometer with 405 nm laser

Caution

No data are available addressing the mutagenicity or toxicity of F2N12S (Component A) or the SYTOX®AADvanced™ dead cell stain (Component B). Since SYTOX® AADvanced™ dead cell stain binds to nucleic acids, treat the stain as a potential mutagen and use with appropriate care. DMSO is known to facilitate the entry of organic molecules into tissues; handle the DMSO dye solutions with particular caution. Always wear suitable protective clothing, gloves, and eye/face protection when handling these reagents. Dispose of the reagents in compliance with all pertaining local regulations.

Preparing Reagents

1.1 Remove the vial containing the SYTOX® AADvanced™ dead cell stain (Component B) and the vial of DMSO (Component C) from the freezer, and allow the contents to equilibrate to room temperature.

1.2 To prepare a 1 mM SYTOX® AADvanced™ dead cell stain working solution, add 100 µL of DMSO (Component C) to one vial of SYTOX® AADvanced™ dead cell stain (Component B). Mix the solution well.

Stability

The F2N12 solution and the SYTOX® AADvanced™ dead cell stain solution are in DMSO and may be subjected to many freeze-thaw cycles without reagent degradation. Both solutions are stable for 1 year when stored at ≤–20°C.

Experimental Protocols

Flow Cytometry

We have optimized this protocol using Jurkat cells (human T-cell leukemia) stimulated with camptothecin to induce apoptosis. Some modification may be required for other cell types.

2.1 Induce apoptosis in cells using the desired method. Prepare a negative control by incubating cells in the absence of apoptosis inducing agent.

2.2 Harvest the cells after the incubation period and wash them twice in Hanks Balanced Salt Solution (HBSS) or other protein free buffer. Resuspend the cells to a final concentration of 1×10^6 cells/ml in HBSS.

Note: Buffers that contain protein (e.g., BSA, FBS, etc.) have been shown to dramatically reduce F2N12S signal and should not be used.

2.3 Prepare flow cytometry tubes, each containing 1 mL of cell suspension.

2.4 Add 1 µL of F2N12S (Component A) solution in DMSO to each flow cytometer tube and mix well. The final labeling concentration of the stain is 200 nM.

2.5 Add 1 µL of SYTOX® AADvanced™ dead cell stain solution in DMSO to each flow cytometry tube and mix well. The final labeling concentration of the stain is 1 µM.

2.6 Incubate the samples at room temperature for 5 minutes before analysis, **protected from light**.

2.7 Analyze the samples on a flow cytometer with 405 nm excitation, and collect emission with 585/42 and 530/30 bandpass or equivalent for the F2N12S reagent. Analyze the SYTOX® AADvanced™ dead cell stain with 488 nm excitation, and collect emission with a 695/40 bandpass or equivalent.

Note: Perform the analysis within 30 minutes of staining to avoid loss of signal and further cell death.

Setting Up a Ratio Parameter

If you are using the BD FACSDiva™ software, you can easily set up a ratio parameter as follows:

After selecting the appropriate violet channels to collect the 585 and 530 nm emissions in linear mode, select the ratio tab in the cytometer setup window (where parameters,

threshold, and compensation are set). After adding a ratio parameter in this tab, make sure to divide the orange fluorescence channel (585 nm) by the green fluorescence channel (530 nm), selecting the detectors you chose to collect F2N12S emissions. Keep the default scaling factor set at 25. Select the ratio you have set up as a distinct parameter on your acquisition plots just as you would for a standard parameter.

For other instruments or second party analysis software, you generally need to calculate this parameter using parameter math or a derived parameter function. Refer to the vendor's instructions on how to set up such a function.

Imaging

F2N12S may be used in imaging applications. If you are interested in using this reagent for imaging, Shynkar et al.[4] provide a detailed discussion of this topic.

References

1. Cytometry 31, 1 (1998); **2.** J Immunol 148, 2207 (1992); **3.** J Immunol 151, 4274 (1993); **4.** J Am Chem Soc 129, 2187 (2007).

Product List **Current prices may be obtained from our website or from our Customer Service Department.**

Cat. no.	Product Name	Unit Size
A35137	Violet Ratiometric Membrane Asymmetry Probe/Dead Cell Apoptosis Kit *for flow cytometry* *100 assays*	1 kit
Related Products		
A13199	annexin V, fluorescein conjugate (FITC annexin V) *100 assays*	500 µL
A13201	annexin V, Alexa Fluor® 488 conjugate *100 assays*	500 µL
A23204	annexin V, Alexa Fluor® 647 conjugate *100 assays*	500 µL
A35110	annexin V, allophycocyanin conjugate (APC annexin V) *50 assays*	250 µL
A35111	annexin V, R-phycoerythrin conjugate (R-PE annexin V) *50 assays*	250 µL
A35122	annexin V, Pacific Blue™ conjugate *for flow cytometry* *100 assays*	500 µL
M34151	MitoProbe™ $DiIC_1(5)$ Assay kit for flow cytometry *100 assays*	1 mL
M34152	MitoProbe™ JC-1 Assay kit for flow cytometry *100 assays*	1 mL
S7020	SYTOX® Green nucleic acid stain *5 mM solution in DMSO*	250 µL
S34857	SYTOX® Blue dead cell stain *for flow cytometry* *1000 assays* *1 mM solution in DMSO*	1 mL
S34859	SYTOX® Red dead cell stain *for 633 or 635 nm excitation* *5 µM solution in DMSO*	1 mL
T10095	ThiolTracker™ Violet (Glutathione Detection Reagent)	1 set of 3 vials
V35118	Vybrant® FAM Caspase-3 and 7 assay kit *25 assays*	1 kit

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