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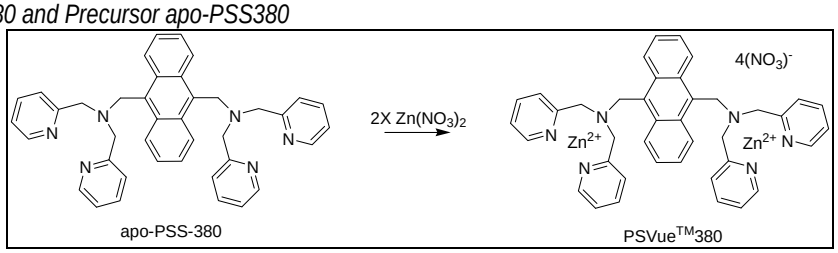
Catalog Number: P-1002

Product Name: PSVue™380, a long wavelength ultraviolet fluorescent probe for detection of apoptotic cells, bacteria and other anionic membranes.

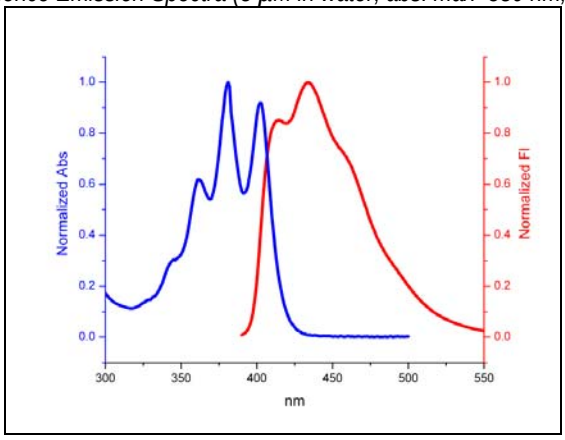
Product Description:
The PSVue™380 (formerly PSS-380) reagent kit contains components to provide a 1 mM solution of PSVue™380 in aqueous solution. The structure and spectral properties of PSVue 380 are shown in Figures 1 and 2 respectively. PSVue380 was originally developed as a sensor for phosphate groups [1] but has also been found to bind strongly to phosphatidylserine (PS) residues exposed on membrane surfaces, through its zinc(II)-dipicolylamine (Zn-DPA) functionality, thus making it a useful reagent for detection of apoptosis in different cell lines [2, 3, 4, 5], as well as for monitoring PS levels in other cellular processes [6]. Staining selectivity is very similar to that obtained with annexin V-FITC since the Zn-DPA PS affinity group has been shown to bind to the same membrane sites as annexin V [2]. Microscopy data indicate that PSVue380 staining is more intense than annexin V, which is attributed to annexin V's much larger size per fluorophore [2]. Negatively charged bacterial cell walls of both Gram-positive and Gram-negative bacteria (e.g. *S. aureus, E. coli*) have also been shown to be labeled selectively with PSVue380 [7] even in the presence of mammalian cells. Given the ubiquitous presence of anionic membranes in bacteria, PSVue380 is expected to label most strains. An attractive feature of PSVue380 is that its fluorescence emission increases by almost 10-fold upon binding to a bilayer membrane [7] thus eliminating the need to wash the cells after addition of the fluorophore. In addition to its utility in cell biology research, PSVue380 may be useful in the automation of biotechnology processes and high-throughput screening systems for drug candidates.

Figure 1. Structure of PSVue380 and Precursor apo-PSS380



PSVue™ 380 Chemical Data: Molecular Formula $C_{40}H_{36}N_{10}O_{12}Zn_2$; Molecular Weight: 979.6 g/mol;
Extinction coefficient: 1.69×10^4 M^{-1} cm^{-1}(water). Quantum yield: 0.50 (water)

Figure 2. PSVue380 Absorption and Fluorescence Emission Spectra (5 µM in water; abs. max=380 nm; fl.em max=440 nm).



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Kit Components:

- Vial containing pre-weighed amount of apo-PSS380 solid dye (at least 0.5 mg)
- Vial of 8.4 mM zinc nitrate solution in water (0.5 mL)

Note: absolute ethanol is required to formulate the product but is not provided.

Storage/Stability:

- For long term storage, the kit maybe refrigerated at 4-8^{o}C. Bring to room temperature before use.
- Once formulated the PSVue 380 dye stock must be protected from bright direct light and examined for crystals prior to use. If crystals are noted in the dye stock, it can be warmed slightly to 40^{o}C in a water bath and sonicated or vortexed to redissolve the crystals.
- The PSVue 380 2 mM stock solution should be stored at 4 $^{\circ}$C and is best used within 5 days.

Formulation Procedure to Prepare 2 mM stock solution:

1. Using pre-weighed apo-PSS-380 solid supplied, prepare a 4mM solution of apo-PSS-380 (i.e. 2.404 mg/mL) in absolute ethanol in the 2mL vial. [Note: Make sure the solid is fully dissolved. Sonication for about 20 minutes is necessary].
2. Add an equal volume of the 8.4mM zinc nitrate solution in water provided to the apo-PSS-380 solution from step 1.
3. Sonicate and/or heat the solution in a water bath at 40^{0}C for 30 minutes to provide a homogeneous solution.
4. Label the vial as **2.0 mM PSVue 380 stock solution in 1:1 ethanol/water**.

In Vitro Cell Staining Conditions:

1. Typical concentrations of PSVue 380 used for *in vitro* cell labeling studies are in the range of 1-50 µM.
2. The recommended buffer for cell staining is a TES [N-tris-(hydroxymethyl)-methyl-2-aminoethane sulfonic acid] buffer system comprising (5 mM TES, 145 mM NaCl, pH=7.4), as used in references [8], [9] and [10].TES buffer should also be used for any wash steps after labeling, if necessary.

Notes: (i) PBS buffer can cause problems with *in vitro* cell staining using PSVue dyes due to the presence of anionic phosphate therefore it should NOT be used.

(ii) PSVue 380 binding to apoptotic cells is almost instantaneous [3].

In Vitro Imaging Conditions:

Fluorescence microscopy of PSVue 380 label cells can be performed on a fluorescent microscope with excitation at 350nm and a DAPI/Hoechst/AMCA filter set [2]. Flow cytometry of labeled cells has been performed with an Enterprise II laser with excitation at 350nm and emission collected at 440nm [2].

References:

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This product is offered for research purposes only and is not intended for human therapeutic or diagnostic use.

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