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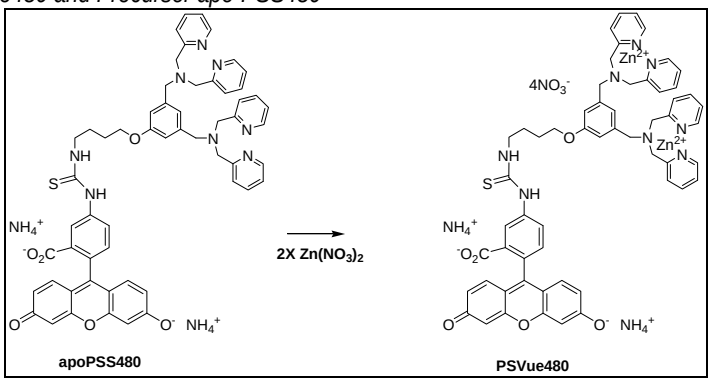
Catalog Number: P-1003

Product Name: PSVue™480, a visible fluorescent probe for detection of apoptotic cells, bacteria and other anionic membranes.

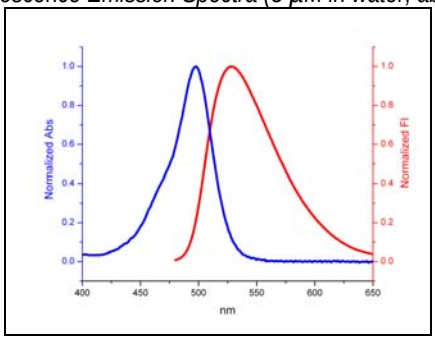
Product Description:
The PSVue™480 (formerly PSS-480) reagent kit contains components to provide a 1 mM solution of PSVue™480 in aqueous solution. The structure and spectral properties of PSVue 480 are shown in Figures 1 and 2 respectively. PSVue480 has been found to bind strongly to the phosphatidylserine (PS) residues exposed on the cell surface of apoptotic cells, through its zinc(II)-dipicolylamine (Zn-DPA) functionality, making it a useful apoptosis sensor [1, 2]. 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 [1], thus making it a more cost effective alternative to FITC labeled Annexin V in various cell death assays. Negatively charged bacterial cell walls are also expected to be labeled selectively with PSVue 480 [3, 4] (e.g. *S. aureus, E. coli*). In addition, tumor cells such as glioma cells which express increased levels of PS on their surface can be labeled with this probe [5]. In addition to its utility in cell biology research, PSVue 480 may be useful in the automation of biotechnology processes and high-throughput screening systems for drug candidates.

Figure 1. Structure of PSVue480 and Precursor apo-PSS480



PSVue™ 480 Chemical Data: Molecular Formula $C_{57}H_{58}N_{14}O_{18}SZn_2$; Molecular Weight: 1390 g/mol; Extinction coefficient: 5.61×10^4 M^{-1} cm^{-1}(in 50:50 DMSO/water at 504 nm).

Figure 2. PSVue480 Absorption and Fluorescence Emission Spectra (5 µM in water; abs. max=480 nm; fl.em max=519 nm).



Kit Components:

- Vial containing pre-weighed amount of apo-PSS480 solid dye (at least 0.5 mg)
- Vial of 4.2mM zinc nitrate solution in water (0.5 mL)

Note: DMSO 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 PSVue480 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 PSVue480 1 mM stock solution should be stored at 4 $^{\circ}$C and is best used within 5 days.

Formulation Procedure to Prepare 1mM stock solution:

1. Using pre-weighed apo-PSS480 solid supplied, prepare a 2 mM solution of apo-PSS480 (i.e. 2.02mg/mL) in DMSO in the 2 mL vial. [Note: Make sure the solid is fully dissolved]
2. Add an equal volume of 4.2 mM zinc nitrate solution provided to the apo-PSS480 from step 1.
3. Place the solution in a water bath at 40^{o}C and shake frequently for 30 minutes to ensure complete complexation.
4. A clear orange colored solution of 1 mM PSVue480 should be obtained. Label as 1 mM PSVue480 stock solution in 1:1 DMSO/water.
5. Keep solution from step 4 in a water bath at 37-40^{o}C until use.

In Vitro Cell Staining Conditions:

1. Typical concentrations of PSVue480 used for *in vitro* cell labeling studies are in the 5-10µM range [1].
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 [1], [3] and [6].TES buffer should also be used for any wash steps after labeling.

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) The presence of up to 10% serum in the binding medium has been shown to have no adverse effect on staining, and apoptotic cells were successfully labeled with PSVue480 from 4-37^{o}C with incubation times as short as 30 seconds [1].
(iii) Staining of fixed cells has not be demonstrated.

In Vitro Imaging Conditions:

Fluorescence microscopy of PSVue480 label cells can be performed on a fluorescent microscope with equipped with FITC/RSGFP/Bodipy/Fluo3/DiO filter sets [1]. Flow cytometry of labeled cells can be performed using an argon laser and 520nm bandpass filter [1].

References:

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3. Leevy, W. M.; Gammon, S. T.; Jiang, H.; Johnson, J. R.; Maxwell, D. J.; Marquez, M.; Piwinica-Worms, D.; Smith, B. D. *Optical imaging of bacterial infection in living mice using a fluorescent near-infrared molecular probe*. J. Am. Chem. Soc. **2006**, *128*, 16476-16477.
4. Leevy, W. M.; Gammon, S. T.; Johnson, J. R.; Lampkins, A. J.; Jiang, H.; Marquez, M.; Piwinica-Worms, D.; Smith, B. D. *Noninvasive optical imaging of staphylococcus aureus bacterial infection in living mice using a bis-dipicolylamine-zinc (II) affinity group conjugated to a near-infrared fluorophore*. Bioconjugate Chem. **2008**, *19*, 686-692.
5. Dr Xiaoyang Qi, Cincinnati Children's Hospital Medical Center, personal communication.

6. DiVittorio KM, Johnson JR, Johansson E, Reynolds AJ, Jolliffe KA and Smith BD. *Synthetic peptides with selective affinity for apoptotic cells*. Org. Biomol. Chem. 2006, 4, 1966-1976.

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

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