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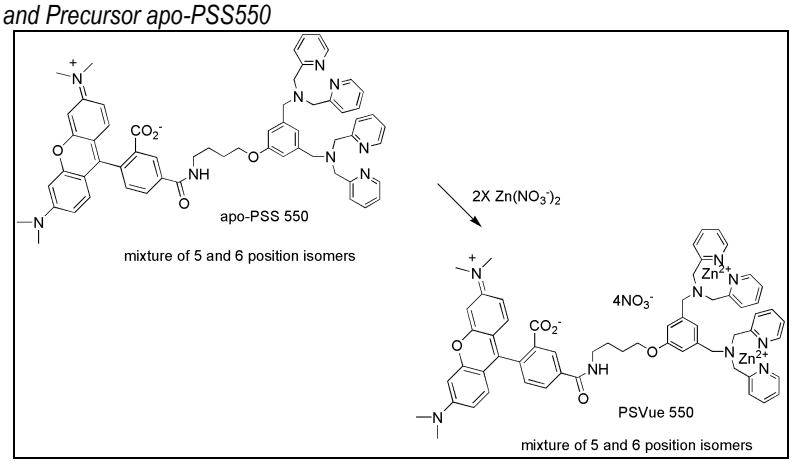
Catalog Number: P-1005

Product Name: PSVue™550, a visible fluorescent probe for detection of apoptotic cells, bacteria and other anionic membranes.

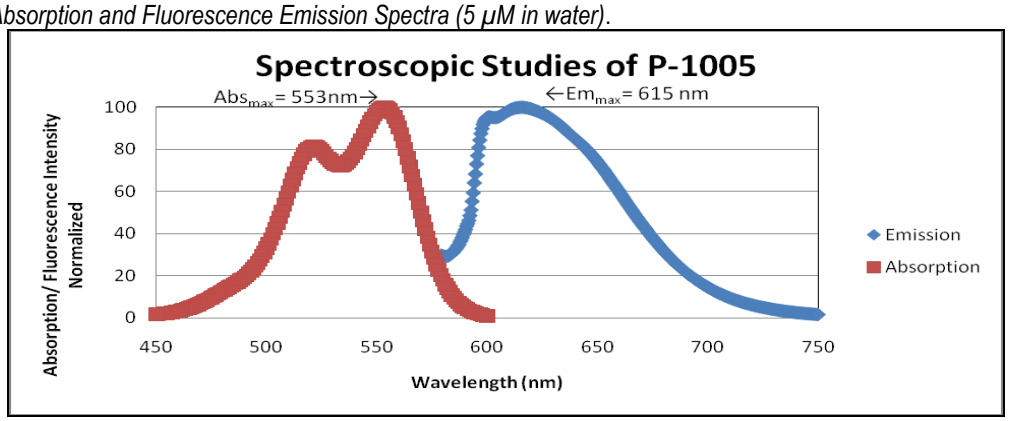
Product Description:
The PSVue™550 reagent kit contains components to provide a 1 mM solution of PSVue™550 in aqueous solution. The structure and spectral properties of PSVue 550 are shown in Figures 1 and 2 respectively. Like other members of the PSVue family of reagents [1-6], PSVue 550 is expected 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. Negatively charged bacterial cell walls are also expected to be labeled selectively with PSVue 550. In addition to its utility in cell biology research, PSVue 550 may be useful in the automation of biotechnology processes and high-throughput screening systems for drug candidates.

Figure 1. Structure of PSVue550 and Precursor apo-PSS550



PSVue™ 550 Chemical Data: Molecular Formula $C_{60}H_{61}N_{13}O_{17}Zn_2$; Molecular Weight: 1365 g/mol;

Figure 2. PSVue550 Absorption and Fluorescence Emission Spectra (5 µM in water).



Kit Components:

- Vial containing pre-weighed amount of apo-PSS550 solid dye (at least 0.5 mg)
- Vial of 10.5mM 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°C. Bring to room temperature before use.
- Once formulated the PSVue550 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°C in a water bath and sonicated or vortexed to redissolve the crystals.
- The PSVue550 1 mM stock solution should be stored at 4 ^{o}C and is best used within 5 days.

Formulation Procedure to Prepare 1mM stock solution of PSVue550:

1. Using pre-weighed apo-PSS550 solid supplied, prepare a 5mM solution (i.e. 5mg/mL) in DMSO in the 2 mL vial. [Note: Make sure the solid is fully dissolved]
2. Add an equal volume of 10.5 mM zinc nitrate solution provided to the apo-PSS550 from step 1 to provide a 2.5mM solution of PSVue550
3. Shake frequently for 30 minutes to ensure complete complexation.
4. Make a 2.5-fold dilution with water to provide a 1mM stock solution.
5. A clear red colored solution should be obtained. Label as 1 mM PSVue550 stock solution in water containing 20% DMSO.

In Vitro Cell Staining Conditions:

1. Typical concentrations of PSVue550 used for *in vitro* cell labeling studies are expected to be 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.

References:

1. Hanshaw RG, Lakshmi C, Lambert TN, Johnson JR and and Smith BD. *Fluorescent detection of apoptotic cells by using zinc coordination complexes with a selective affinity for membrane surfaces enriched with phosphatidylserine*. ChemBioChem 2005, 12, 2214-2220.
2. Hanshaw RG and Smith BD. *New reagents for phosphatidylserine recognition and detection of apoptosis*. Bioorg. & Med. Chem. 2005, 13, 5035-5042
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.

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