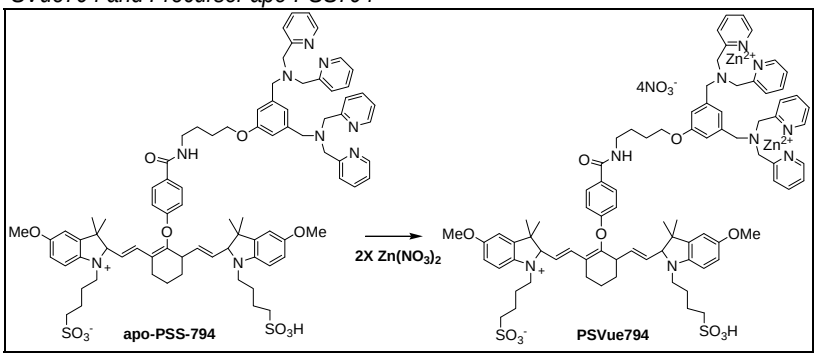
www.mtarget.com ● tel:610 738 7938 ● email: briangray@mtarget.com

Catalog Number: P-1001

Product Name: PSVue™794, a near-infrared fluorescent probe for detection of apoptotic cells, bacteria and other anionic membranes.

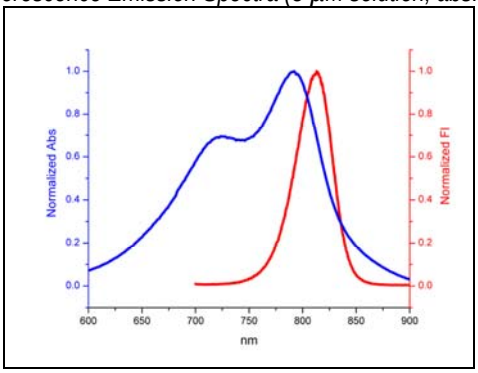
Product Description:
The PSVue™794 (formerly PSS794) reagent kit contains components to provide a 1 mM solution of PSVue™794 in aqueous solution. The structure of PSVue 794 is shown in Figure 1. The compound exhibits absorbance and fluorescence excitation maximum at 794 nm and emission maximum at 810 nm (Figure 2) and through its zinc(II)-dipicolylamine (Zn-DPA) functionality has been found to bind strongly to negatively charged bacterial cell walls [1, 2] (e.g. *S. aureus, E. coli*) and necrotic regions present in various tumors [3] (e.g. mammary, prostate, glioma) *in vitro and in vivo*. In particular, it has also been found to bind to the phosphatidylserine (PS) residues exposed on the cell surface of apoptotic cells making it a more cost effective alternative to fluorescently labeled Annexin V in various cell death assays [4]. The labeling vehicle provided with the kit (Diluent X) is designed to maximize dye solubility and is suitable for *in vitro* and *in vivo* use.

Figure 1. Structure of PSVue794 and Precursor apo-PSS794



PSVue™ 794 Chemical Data: Molecular Formula $C_{83}H_{95}N_{13}O_{23}S_2Zn_2$; Molecular Weight: 1837.6 g/mol;
Extinction coefficient: 1.1×10^5 M^{-1} cm^{-1} (in water);
Quantum yield: 0.14 in water (higher in organic solvent)

Figure 2. PSVue794 Absorption and Fluorescence Emission Spectra (5 µM solution; abs. max=794 nm; fl.em max=810 nm).



Kit Components:

- Vial containing pre-weighed amount of apo-PSS794 solid dye (at least 1 mg)

- Vial of Diluent X (1 mL)
- Vial of 4.2mM zinc nitrate solution in diluent X (1 mL)

Storage/Stability:

- For long term storage, the kit maybe refrigerated at 4-8^{o}C. Bring to room temperature before use.
- Once formulated the PSVue794 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 PSVue794 1 mM stock solution should be stored at 4 °C and is best used within 5 days.

Formulation Procedure to Prepare 1mM stock solution:

1. Using pre-weighed apo-PSS solid supplied, prepare a 2 mM solution of apo-PSS-794 (i.e. 2.92mg/mL) in Diluent X 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-PSS-794 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 green colored solution of 1 mM PSVue794 should be obtained.
5. Keep solution from step 4 in a water bath at 37-40^{o}C until use.
6. Typical doses of PSVue794 for *in vivo* tumor imaging studies are 3-4 mgs/kg [3]. For bacterial imaging in mice a dose of 75uL of 1 mM PSVue794 has been reported [1, 2].

In Vitro Cell Staining Conditions:

1. A typical concentration of PSVue794 used for *in vitro* cell labeling studies is 10µM [1, 3].
2. The recommended buffer for *in vitro* 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], [5] and [6].TES buffer should also be used for any wash steps after labeling.

Note: PBS buffer can cause problems for *in vitro* cell staining using PSVue dyes due to the presence of anionic phosphate therefore it should NOT be used for *in vitro* studies.

In Vitro Imaging Conditions:

Near infrared fluorescence images can be captured using a Photometrics Cascade 512B CCD and a Cy7 filter set (Exciter HQ710/75x, Dichroic Q750LP, Emitter HQ810/90m).

In Vivo Imaging Conditions:

Kodak 4000MM imaging station (or similar) configured for epi-illumination. Illuminate animal with filtered light at either:

(i) 755 ± 20 nm and collect emission fluorescence at 830 ± 10 nm (2, 3),or
(ii) 750 ± 10 nm and collect emission fluorescence at 830 ± 20 nm (3), or
(iii) 720 ± 35 nm and collect image intensity at 790 ± 35 nm by CCD camera during a 60s acquisition period [1, 2].

References:

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4. Hanshaw RG and Smith BD. *New reagents for phosphatidylserine recognition and detection of apoptosis*. Bioorg. & Med. Chem. 2005, 13, 5035-5042
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This product is offered for research purposes only and is not intended for human therapeutic or diagnostic use.

833 Lincoln Avenue, Unit 9, West Chester, PA 19380 USA www.mtarget.com
Tel: 610-738-7938 Fax: 610-738-7928 E-mail: briangray@mtarget.com Version 2: May 2010