

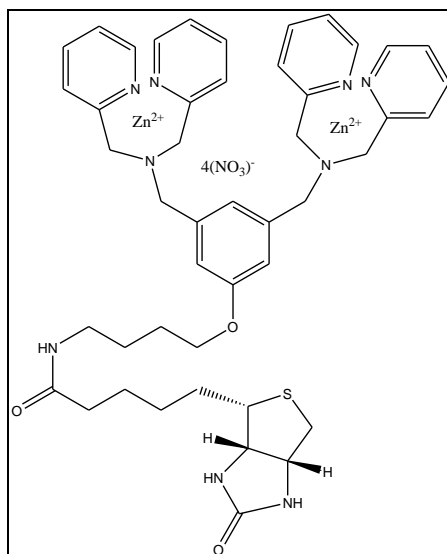
Catalog Number: P-1004

Product Name: PSVue™ Biotin, a biotinylated probe that binds to apoptotic cells, bacteria and other anionic membranes.

Product Description:

The structure of PSVue™ Biotin is shown in Figure 1. Through its zinc(II)-dipicolylamine (Zn-DPA) moiety it is expected to be capable of binding to range of anionic phospholipid membranes in the same way that fluorescent versions containing the Zn-DPA motif bind [1-6]. The biotin moiety provides a detection handle by being able to form complexes with avidin or streptavidin labeled materials for a variety of biological applications. In one application, a complex comprising PSVue biotin bound to streptavidin quantum dots was formed and shown to distinguish between E. coli mutants and permit *in vivo* imaging of bacteria [7].

Figure 1. Structure of PSVue Biotin



PSVue™ Biotin Chemical Data: Molecular Formula $C_{46}H_{55}N_{13}O_{15}SZn_2$; Molecular Weight: 1191 g/mol

Kit Components:

- Vial containing pre-weighed amount of PSVue Biotin solid (at least 1 mg)

Storage/Stability:

- For long term storage, the solid may be refrigerated at 4-8°C. Bring to room temperature before use.
- The PSVue Biotin 0.5 mM stock solution should be stored at 4 °C and is best used within 5 days.

Procedure to Prepare 0.5mM stock solution:

1. Prepare a TES [N-tris-(hydroxymethyl)-methyl-2-aminoethanesulfonic acid] buffer solution (pH 7.4) as follows: dissolve 114.6 mg of TES and 848 mg of NaCl in 100 mL of DI water and bring the pH to 7.4 with 2N NaOH. This gives a 5 mM TES (1.146 mg/mL) and 145 mM NaCl (8.48 mg/mL) solution.

- Using pre-weighed PSVue Biotin solid supplied, prepare a 0.5 mM solution of PSVue Biotin (i.e. 0.60 mg/mL) in TES buffer in the 2 mL vial. [Note: Make sure the solid is fully dissolved, sonicate to make the solution homogeneous].
- A clear solution of 0.5 mM PSVue Biotin should be obtained.

Typical Procedure to Prepare PSVue Biotin Streptavidin Quantum Dot Complex

- Incubate 10 μ L of 40 μ M PSVue Biotin in TES buffer (5 mM TES, 145 mM NaCl, pH 7.4) for 10 minutes with 100 μ L of the 1 μ M stock solution of the streptavidin-coated quantum dot of choice. **GQD** (Qdot[®] 565 Streptavidin Conjugate, em: 565 nm, Invitrogen, Q10101MP), **RQD** (Qdot[®] 655 Streptavidin Conjugate, em: 665 nm, Invitrogen, Q10121MP), or **NIRQD** (Qdot[®] 800 Streptavidin Conjugate, em: 800 nm, Invitrogen, MP10171MP) can be used as well as any comparable Quantum Dots.
- This 4 μ M PSVue Biotin Streptavidin Quantum Dot complex can then be used to label bacteria.

In Vitro Cell Staining Conditions:

- Pellet 0.5 mL of bacteria with an O.D. of 0.5. Carefully remove the supernatant with a pipettor. Resuspend the pellet in the 110 μ L of the PSVue Biotin Streptavidin Quantum Dot complex solution and incubate for 15 minutes.
- After incubation, wash the bacteria by adding 500 μ L of the TES buffer and centrifuging. Remove the supernatant. Repeat the wash step. Add another 500 μ L of the TES buffer and use this suspension for imaging.

In Vivo Mouse Conditions:

- The PSVue Biotin Streptavidin Quantum Dot complex solution can be used to image mice.
- The typical dose used for *in vivo* bacterial imaging in mice is 50 μ L of the 4 μ M PSVue Biotin Streptavidin Quantum Dot solution cell suspension.

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:

Fluorescence images can be captured using a Nikon Eclipse TE2000-U epifluorescence microscope and a filter setting appropriate for the Quantum Dot used. For the **GQD** (Qdot[®] 565 Streptavidin Conjugate, em: 565 nm, Invitrogen, Q10101MP), a “green” filter set can be used (Exciter: D480/30X, Dichroic: 400DCLP, Emitter: HQ535/50m). For the **RQD** (Qdot[®] 655 Streptavidin Conjugate, em: 665 nm, Invitrogen, Q10121MP), a “red” filter set can be used (Exciter: HQ545/30x, Dichroic: Q570LP, Emitter: HQ610/75m). For the **NIRQD** (Qdot[®] 800 Streptavidin Conjugate, em: 800 nm, Invitrogen, MP10171MP), a “near infrared” filter set can be used (Exciter: HQ710/75x, Dichroic: Q750LP, Emitter: HQ810/90m).

In Vivo Imaging Conditions:

IVIS Lumina *in vivo* imaging station (or similar). Illuminate animal with light. For the **NIRQD** (Qdot[®] 800 Streptavidin Conjugate, em: 800 nm, Invitrogen, MP10171MP), a Cy 5.5 exciter (635 \pm 20 nm) and ICG emitter (840 \pm 30 nm) can be used with a 10 s acquisition time, Fstop = 1, and low binning (2x2). The image must then be background subtracted and set to “Fire” fluorescence intensity scale using ImageJ v1.37 software.

References:

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