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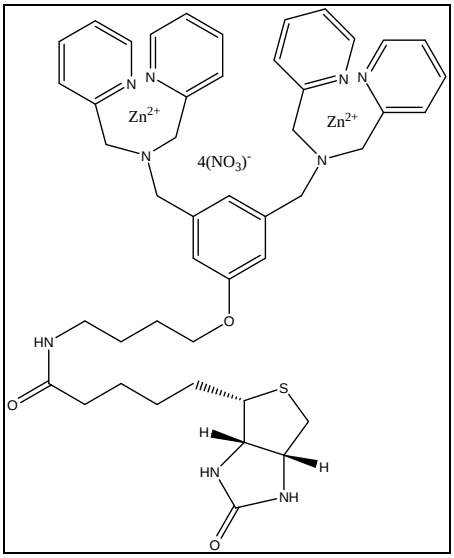
Catalog Number: P-1004

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 maybe 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.

2. 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].
3. A clear solution of 0.5 mM PSVue Biotin should be obtained.

Typical Procedure to Prepare PSVue Biotin Streptavidin Quantum Dot Complex

1. Incubate 10 µL of 40 µM PSVue Biotin in TES buffer (5 mM TES, 145 mM NaCl, pH 7.4) for 10 minutes with 100 µL of the 1uM 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.
2. This 4 µM PSVue Biotin Streptavidin Quantum Dot complex can then be used to label bacteria.

In Vitro Cell Staining Conditions:

1. 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 µL of the PSVue Biotin Streptavidin Quantum Dot complex solution and incubate for 15 minutes.
2. After incubation, wash the bacteria by adding 500 µL of the TES buffer and centrifuging. Remove the supernatant. Repeat the wash step. Add another 500 µL of the TES buffer and use this suspension for imaging.

In Vivo Mouse Conditions:

1. The PSVue Biotin Streptavidin Quantum Dot complex solution can be used to image mice.
2. The typical dose used for *in vivo* bacterial imaging in mice is 50 µL of the 4 µM 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 ± 20 nm) and ICG emitter (840 ± 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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