INSTRUCTIONS

NHS/Nitrophenyl Azide Crosslinkers
(ANB-NOS and Sulfo-SANPAH)

21451 22589

0635.4

Number	Description
21451	**ANB-NOS** (*N*-5-azido-2-nitrobenzoyloxysuccinimide), 50mg Molecular Weight: 305.20 Spacer Arm: 7.7Å Mass Added: 162.00
22589	**Sulfo-SANPAH** (sulfosuccinimidyl-6-[4´-azido-2´-nitrophenylamino]hexanoate), 50mg Molecular Weight: 492.40 Spacer Arm: 18.2Å Mass Added: 247.09

Storage: Store ANB-NOS at 4°C. Store Sulfo-SANPAH at -20°C. Store protected from moisture and light. These crosslinkers are shipped at ambient temperature.

Introduction

Thermo Scientific ANB-NOS and Sulfo-SANPAH are heterobifunctional crosslinkers that contain an amine-reactive *N*-hydroxysuccinimide (NHS) ester and a photoactivatable nitrophenyl azide. NHS esters react efficiently with primary amino groups (-NH_2) in pH 7-9 buffers to form stable amide bonds. The reaction results in the release of *N*-hydroxy-succinimide (Figure 1). When exposed to UV light nitrophenyl azides form a nitrene group that can initiate addition reactions with double bonds, insertion into C-H and N-H sites, or subsequent ring expansion to react with a nucleophile (e.g., primary amines). The latter reaction path dominates when primary amines are present (Figure 1).

The water-soluble and water-insoluble forms of NHS-esters have essentially identical reactivity toward primary amines. Sulfo-SANPAH is supplied as a sodium salt, which is water-soluble to a concentration of 10mM. ANB-NOS is water-insoluble and first dissolved in an organic solvent such as DMSO or DMF then added to the aqueous reaction mixture. ANB-NOS does not possess a charged group and is lipophilic and membrane-permeable, which makes it useful for intracellular and intramembrane conjugations. Water-soluble Sulfo-SANPAH possesses charged groups and is useful for cell-surface protein crosslinking.

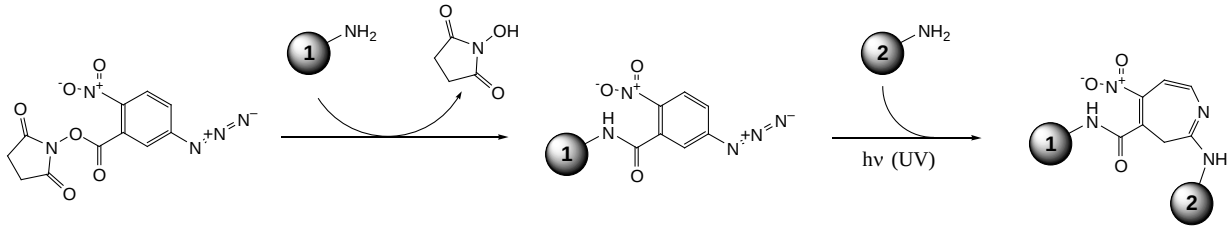

Figure 1. Predominant reaction pathway for ANB-NOS, resulting in a total mass addition of 162.00.

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Important Product Information

- These crosslinkers are moisture-sensitive. To avoid moisture condensation onto the product, equilibrate vial to room temperature before opening. Prepare these crosslinkers immediately before use. The NHS-ester moiety readily hydrolyzes and becomes non-reactive; therefore, do not prepare stock solutions for storage. Discard any unused, reconstituted crosslinker.
- Hydrolysis of the NHS ester is a competing reaction and increases with increasing pH. Hydrolysis occurs more readily in dilute protein or peptide solutions. In concentrated protein solutions, the acylation reaction is favored.
- Use non-amine-containing buffers at pH 7-9 such as 20mM sodium phosphate, 0.15M NaCl (Product No. 28372); 20mM HEPES; 100mM carbonate/bicarbonate; or 50mM borate. Do not use buffers that contain Tris, glycine or sulfhydryls. Tris and glycine will compete with the intended reaction and thiols can reduce the azido group.
- For protein concentration greater than 5mg/mL, use a 10-fold molar excess of the crosslinker. For samples <5mg/mL, use a 20- to 50-fold molar excess of the crosslinker. Use a final concentration of crosslinker at 0.1-10mM.
- Dissolve Sulfo-SANPAH in room-temperature water up to 10mM; solubility decreases with increasing salt concentration. Dissolve ANB-NOS in a dry water-miscible organic solvent such as DMSO or DMF. The percentage of solvent maintained during the crosslinking reaction is typically 1-10% of the final reaction volume; however, the crosslinker may precipitate at >5mM in 1-10% solvent.
- For best results, react the NHS end of the crosslinkers (in the dark) first. After removing the hydrolyzed and non-reacted crosslinker by gel filtration or dialysis, the activated molecule can be coupled to a second molecule by photolysis.

Photolysis (Photoactivation) Information

- For maximum efficiency, use a shallow reaction vessel for photolysis. Irradiation efficiency decreases as the distance light must penetrate through the solution increases. Use a low protein-binding vessel for maximum sample recovery.
- For photolysis use a UV lamp that irradiates at 300-460nm (see **Note** below). High wattage lamps are more effective and require shorter exposure times than low wattage lamps. Suggestions for lamps include the Stratalinker 2400 (5 × 15 watt bulbs, emission at either 312nm or 365nm), mercury vapor lamps (180 watt, 350 watt, from 300 to 360nm), XeCl excimer laser (150mJ, 308nm) and UV Spectroline lamps (medium/long wavelength lamps). Using low wattage hand-held lamps, such as 6 watt lamps, will result in lower conjugation efficiencies.

  Note: Avoid UV lamps that emit light at 254nm; this wavelength causes proteins to photodestruct. Filters that remove light at wavelengths below 300nm are ideal. Using a second filter that removes wavelengths above 370 nm could be beneficial but is not essential.
- Position the UV lamp 5-10cm from the reaction. For lamps >150 watts use a distance of 10cm. For lower powered lamps, use a distance of 5cm. Perform photolysis by placing the lamp above the reaction as the reaction vessel may impede irradiation by filtering some of the UV light.

Please visit our website for additional information relating to this product including the following items:

- Tech Tip #11: Light sources and conditions for photoactivation of aryl azide crosslinking reagents
- Tech Tip #43: Protein stability and storage
- Tech Tip #3: Determine reactivity of NHS-ester biotinylation and crosslinking reagents
- Tech Tip #5: Attach an antibody onto glass, silica or quartz surface

Related Thermo Scientific Products

20036	**Bioconjugate Techniques,** 2nd edition, 785 pages, softcover
28372	**BupH™ Phosphate Buffered Saline Pack,** 40 packs
20290	**DTT (Dithiothreitol),** 5g
20291	**No-Weigh™ DTT (Dithiothreitol),** 48 × 7.7mg microtubes
35602	**2-Mercaptoethanol**
66382, 66807	**Slide-A-Lyzer Dialysis Cassette Kits**

66528 **Slide-A-Lyzer Concentrating Solution,** 200mL

66529 **Slide-A-Lyzer Concentrating Solution,** 500mL

General References

ANB-NOS

Adams, C.A., *et al*. (2004). Self-association of the amino-terminal domain of the yeast TATA-binding protein. *J Biol Chem* **279:**1376-82.

Chang I.N., *et al.* (1995). Photoaffinity labeling of antibodies for applications in homogeneous fluoroimmunoassays. *Anal Chem* **67:**959-66.

Park, B, *et al.* (2003). A single polymorphic residue within the peptide-binding cleft of MHC class I molecules determines spectrum of tapasin dependence. *J Immunol* **170:**961-8.

Sulfo-SANPAH

Gaudet, C., *et al.* (2003). Influence of type I collagen surface density on fibroblast spreading, motility, and contractility. *Biophys. J.* **85:**3329-35.

Uckun F.M., *et al.* (1995). Biotherapy of B-cell precursor leukemia by targeting genistein to CD19-associated tyrosine kinases. *Science* **267:**886-91.

Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.