INSTRUCTIONS

Pierce Sulfo-SANPAH, No-Weigh Format

MAN0017086
Rev. A.0
Pub. Part No. 2162637.0

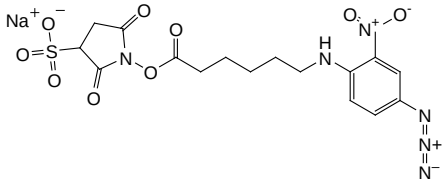
A35395

Number	Description
A35395	**Pierce Sulfo-SANPAH, No-Weigh Format** (sulfosuccinimidyl-6-[4´-azido-2´-nitrophenylamino]hexanoate), 10 × 1mg

Molecular Weight: 492.40

Spacer Arm: 18.2Å

Mass Added: 247.09



For Research Use Only. Not for use in diagnostic procedures.

Storage: Store Sulfo-SANPAH at -20°C. Store protected from moisture and light. Shipped on ice packs.

Introduction

Thermo Scientific™ Pierce™ Sulfo-SANPAH is a heterobifunctional crosslinker that contains 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 Sulfo-*N*-hydroxy-succinimide. 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). Sulfo-SANPAH, supplied as a sodium salt, is useful for cell-surface protein crosslinking and possesses charged groups for water solubility to a concentration of 10mM.

Thermo Scientific™ No-Weigh™ products are specialty reagents provided in a pre-aliquoted format. The pre-weighed packaging prevents the loss of reagent reactivity and contamination over time by eliminating the repetitive opening and closing of the vial. The format enables use of a fresh vial of reagent each time, eliminating the hassle of weighing small amounts of reagents and reducing concerns over reagent stability.

Important Product Information

- Sulfo-SANPAH is moisture-sensitive. To avoid moisture condensation onto the product, equilibrate vial to room temperature before opening. Prepare 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.

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- Dissolve Sulfo-SANPAH in room-temperature water up to 10mM; solubility decreases with increasing salt concentration. Sulfo-SANPAH may also be dissolved in a dry water-miscible organic solvent such as DMSO or DMF. Add 200µL of buffer or solvent to a 1mg vial for a 10mM solution. Mix by repeated pipetting or replace cap and vortex. Higher molarity solutions can be obtained when DMSO or DMF is used rather than an aqueous buffer. The maximum useable volume of the vial is 800µL.
- 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

21451	**ANB-NOS** (*N*-5-azido-2-nitrobenzoyloxysuccinimide), 50mg
22589	**Sulfo-SANPAH** (sulfosuccinimidyl-6-[4´-azido-2´-nitrophenylamino]hexanoate), 50mg
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

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.

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