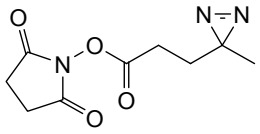
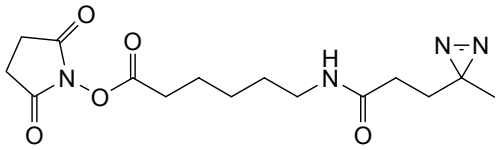
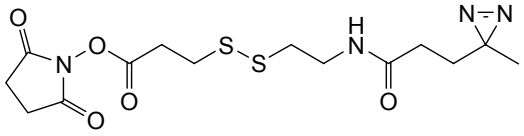
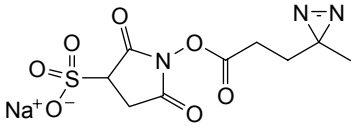
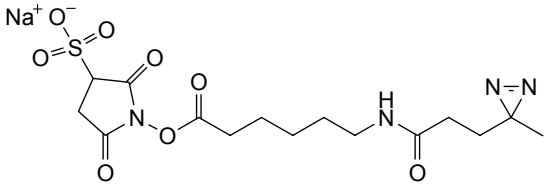
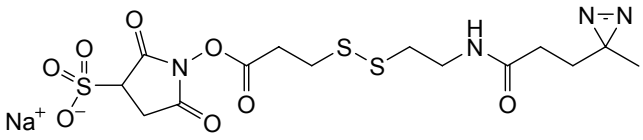
INSTRUCTIONS

Amine-Reactive Diazirine Crosslinkers

2067.0

Number	Description
26167	**NHS-Diazirine (SDA)** (succinimidyl 4,4-azipentanoate), 50 mg Molecular Weight: 225.20 Spacer Arm: 3.9Å 
26168	**NHS-LC-Diazirine (LC-SDA)** (succinimidyl 6-(4,4´-azipentanamido)hexanoate), 50 mg Molecular Weight: 338.36 Spacer Arm: 12.5Å 
26169	**NHS-SS-Diazirine (SDAD)** (succinimidyl 2-[(4,4´-azipentanamido)ethyl]-1,3´-dithioproprionate), 50 mg Molecular Weight: 388.46 Spacer Arm: 13.6Å 
26173	**Sulfo-NHS-Diazirine (Sulfo-SDA)** (sulfosuccinimidyl 4,4´-azipentanoate), 50 mg Molecular Weight: 327.25 Spacer Arm: 3.9Å 
26174	**Sulfo-NHS-LC-Diazirine (Sulfo-LC-SDA)** (sulfosuccinimidyl 6-(4,4´-azipentanamido)hexanoate), 50 mg Molecular Weight: 440.40 Spacer Arm: 12.5Å 
26175	**Sulfo-NHS-SS-Diazirine (Sulfo-SDAD)** (sulfosuccinimidyl 2-[(4,4´-azipentanamido)ethyl]-1,3´-dithioproprionate), 50 mg Molecular Weight: 490.51 Spacer Arm: 13.6Å 

Storage: Store all products at 4°C in dark protected from moisture. Products shipped at ambient temperature.

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Introduction

NHS-ester diazirines (SDA, LC-SDA, SDAD, Sulfo-SDA, Sulfo-LC-SDA and Sulfo-SDAD) are heterobifunctional crosslinkers that contain an amine-reactive *N*-hydroxysuccinimide (NHS) ester and a photoactivatable diazirine ring. 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 NHS (Figure 1). Photoactivation of diazirines with long-wave UV light creates reactive carbene intermediates that can form covalent bonds through addition reactions with any amino acid side chain or peptide backbone within the spacer arm distance.[1,2]

SDA, LC-SDA and SDAD are water-insoluble crosslinkers that are dissolved in an organic solvent, such as DMSO or DMF, before adding to the aqueous reaction mixture. These NHS-ester diazirine derivatives do not possess a charged group and are membrane-permeable, which makes them useful for intracellular and intramembrane conjugations. Sulfo-SDA, Sulfo-LC-SDA and Sulfo-SDAD are water-soluble, NHS-ester diazirine derivatives and are supplied as sodium salts. Sulfo-NHS diazirine derivatives possess negatively charged sulfate groups that reduce cell membrane permeability, making these crosslinkers useful for cell-surface protein crosslinking.

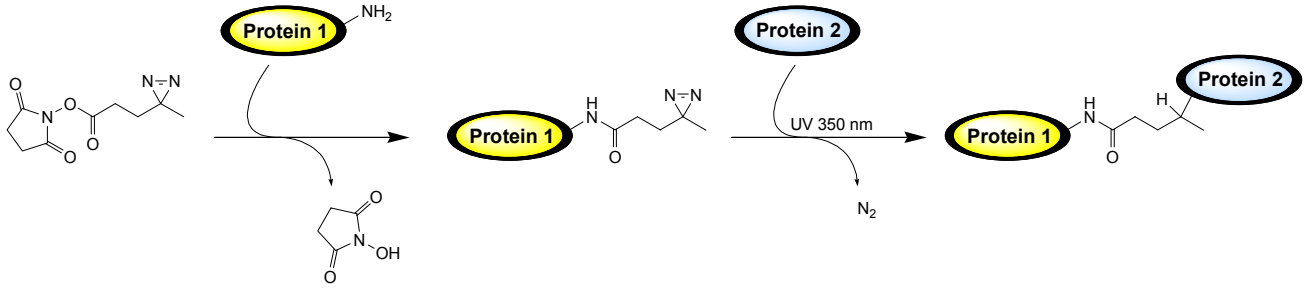

Figure 1. Schematic of the NHS-diazirine crosslinking reaction.

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 competes with crosslinking primary amines of proteins/peptides (acylation). Acylation is favored near neutral pH (6-9) and with concentrated protein solutions.
- Use non-amine-containing buffers at pH 7-9 such as PBS (100 mM sodium phosphate, 150 mM NaCl, pH 7.4) (Product No. 28372); 20 mM HEPES; 100 mM carbonate/biocarbonate; or 50 mM borate. Do not use buffers that contain primary amines such as Tris or glycine which compete with acylation.
- Dissolve NHS-ester diazirine derivatives (SDA, LC-SDA, SDAD) in a dry water-miscible organic solvent, such as DMSO and DMF, before diluting to < 10% solvent in final aqueous reaction buffer. NHS-ester diazirine crosslinkers may precipitate if added directly to aqueous buffer at concentrations > 5 mM.
- Dissolve Sulfo-NHS-ester diazirines (Sulfo-SDA, Sulfo-LC-SDA, Sulfo-SDAD) in water up to 10 mM; solubility decreases with increasing salt concentration. Sulfo-NHS-ester diazirine crosslinkers are also soluble in DMSO up to 10 mM.
- To cleave NHS-SS-Diazirine (SDAD) and Sulfo-NHS-SS-Diazirine (Sulfo-SDAD) use 10-50 mM DTT at 37°C for 30 minutes or 5% 2-mercaptoethanol in SDS-PAGE sample buffer (2% SDS, 6.25 mM Tris base, 10% glycerol) at 100°C for 5 minutes.
- For best results, first react the NHS end of the crosslinkers and remove the hydrolyzed and non-reacted crosslinker after quenching by gel filtration or dialysis. The activated molecule can be coupled to a second molecule by photoactivation.

Photoactivation Information

- Use a UV lamp that irradiates between 320-370 nm (see **Note** below) for photoactivation. 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 365 nm), mercury vapor lamps (200 watt, between 300 nm and 360 nm), and Spectroline or UVP hand-held lamps (8 watt, emission at 365 nm). Using lower-wattage hand-held lamps, such as 6 watt lamps, will result in lower crosslinking efficiencies.

Note: The optimal wavelength for diazirine photoactivation is 345 nm.[1] Do not use UV lamps that emit light at 254 nm as this wavelength causes proteins and DNA to photodestruct. Filters that remove light at wavelengths below 300 nm are necessary for mercury vapor lamps.

- Perform UV irradiation in a shallow, uncovered reaction vessel/plate for maximum efficiency. Irradiation efficiency decreases logarithmically with increased distance from the light source. Position a UV lamp 3-5 cm from cells for 15 watt lamps. For lower-powered, hand-held lamps, use a distance of 1 cm without filter, if possible. For lamps > 150 watts, use a distance of 20 cm with a 300 nm filter. Perform photoactivation by placing the lamp above the open reaction vessel to avoid impeding irradiation by the vessel. Samples may need to be rotated for even UV irradiation. Total UV irradiation time should be less than 15 minutes for crosslinking of live cells.

Procedure for *In vitro* Protein Crosslinking

The following protocol is an example application for this product. Specific applications will require optimization.

A. Additional Materials Required

- Dry dimethyl sulfoxide (DMSO) for use with NHS-ester diazirines (SDA, LC-SDA, SDAD)
- Phosphate-buffered saline (PBS, 0.1 M sodium phosphate, 0.15 M sodium chloride; pH 7.2, Product No. 28372)
- Quenching Buffer: 1 M Tris•HCl, pH 8.0
- Zeba™ Spin Desalting Columns or Slide-A-Lyzer® Dialysis Cassettes
- UV lamp with 365 nm bulb(s)

B. Procedure

1. Prepare proteins in PBS.
2. Prepare crosslinker solution immediately before use at 10 mM. Dissolve NHS-ester diazirines stock solutions in DMSO or DMF. Dissolve Sulfo-NHS-ester diazirines in water or PBS. Examples of preparations are as follows:

Table 1. Recommended 10 mM stock solution preparation.

Crosslinker	Molecular Weight (g/mol)	Solvent Volume for 2 mg	Solvent Type
SDA	225.20	888 µl	DMSO or DMF
LC-SDA	338.36	591 µl	DMSO or DMF
SDAD	388.46	515 µl	DMSO or DMF
Sulfo-SDA	327.25	611 µl	Water or PBS
Sulfo-LC-SDA	440.40	454 µl	Water or PBS
Sulfo-SDAD	490.51	408 µl	Water or PBS

3. Add crosslinker to the protein sample at a final crosslinker concentration of 0.5-2 mM. If the protein concentration is ≥ 5 mg/ml, use a 10-fold molar excess of the crosslinker. For samples < 5 mg/ml, use a 20- to 50-fold molar excess of the crosslinker.
4. Incubate the reaction at room temperature for 30 minutes or on ice for 2 hours.
5. Stop the reaction by adding Quenching Buffer to a final concentration of 50-100 mM Tris.
6. Incubate the reaction at room temperature for 5 minutes or on ice for 15 minutes.
7. Remove excess un-reacted crosslinker using Zeba Spin Desalting Columns or Slide-A-Lyzer Dialysis Cassettes.
8. Photoactivate diazirine-labeled proteins using UV lamp with 365 nm bulbs to photocrosslink proteins.

Procedure for Intra- and Extra-cellular Protein Crosslinking

Crosslinking may be performed on cells in suspension or on adherent cells in culture plates. In the latter situation, diffusion of the crosslinking reagent to all cell surfaces will be limited and will occur predominately on the exposed surface. Culture media must be washed from the cells otherwise amine-containing components will quench the reaction. Using a more concentrated cell suspension is most effective as less reagent will be required in the reaction.

A. Additional Materials Required

- Dry dimethyl sulfoxide (DMSO) for use with NHS-ester diazirines (SDA, LC-SDA, SDAD)
- Phosphate-buffered saline (PBS, 0.1 M sodium phosphate, 0.15 M sodium chloride; pH 7.2, Product No. 28372)
- Quenching Buffer: 1 M Tris•HCl, pH 8.0
- UV lamp with 365 nm bulb(s)

A. Procedure

1. Remove media from 10^7cells and wash twice with ice cold PBS.
2. Prepare crosslinker solutions immediately before use. Dissolve NHS-ester diazirines in DMSO or DMF. Dissolve Sulfo-NHS-ester diazirines in PBS.
3. Add a final crosslinker concentration (diluted in PBS) of 0.5-2 mM to the cells.
4. Incubate the reaction at room temperature for 10 minutes or on ice for 30 minutes.
5. Stop the reaction by adding Quenching Buffer to a final concentration of 50-100 mM Tris.
6. Incubate the reaction at room temperature for 5 minutes or on ice for 15 minutes.
7. Remove excess non-reacted crosslinker from cells and rinse twice with PBS.

   Note: Add PBS to completely cover cells to prevent cells from drying during UV irradiation.
8. Position cells 1-5 cm from the UV bulbs and irradiate for 5-15 minutes depending on the number and wattage of 365 nm UV bulbs.
9. Harvest and lyse cells for analysis of crosslinked proteins.

Troubleshooting

Problem	Possible Cause	Solution
Minimal or no crosslinking observed	NHS-ester hydrolysis	Allow product to equilibrate to room temperature before opening
		Make certain DMSO (or DMF) used to prepare stock solution is dry (e.g., anhydrous, molecular sieve-treated)
	Inappropriate conjugation buffer	Avoid buffers that contain primary amines such as Tris or glycine
	Inappropriate molar excess of product to target	Optimize product-to-target ratio – use 20-25 molar excess for 2 mg/ml protein or 10-15 molar excess for 10 mg/ml protein
	Excess crosslinker not quenched and/or removed	Use desalting/dialysis to remove non-derivatized crosslinker before photo-crossslinking
		Rinse cells thoroughly with PBS before photo-crossslinking
	UV light source problem	Use UV wavelengths 320-370 nm for maximal photoactivation – avoid using UV < 300 nm
		Increase time of UV irradiation
		Decrease distance to UV bulbs
		Use multiple UV bulbs with > 8 W output and/or remove UV filters

Related Products

28372	**BupH™ Phosphate Buffered Saline Pack,** 40 packs
20290	**DTT (Dithiothreitol),** 5 g
20291	**No-Weigh™ DTT (Dithiothreitol),** 48 × 7.7 mg microtubes
77720	**TCEP Bond-Breaker Solution,** 5 ml
35602	**2-Mercaptoethanol,** 10 × 1 ml ampules
66382	**Slide-A-Lyzer Dialysis Cassette Kit,** for 0.5-3 ml sample volumes
66807	**Slide-A-Lyzer Dialysis Cassette Kit,** for 3-12 ml sample volumes
89889	**Zeba Spin Desalting Columns, 2 ml,** 5 columns, for 200-700 µl samples volumes
89891	**Zeba Spin Desalting Columns, 5 ml,** 5 columns, for 600-2,000 µl sample volumes
22615	**L-Photo-Methionine,** 100 mg
22610	**L-Photo-Leucine,** 100 mg

References

1. Suchanek, M., *et al.* (2005). Photo-leucine and photo-methionine allow identification of protein-protein interactions. *Nat. Methods*. **2(4)**: 261-8.
2. Yoshihito and Kohler (2008). Photoactivatable crosslinking sugars for capturing glycoprotein interactions. *JACS*. 10.1021/ja7109772.