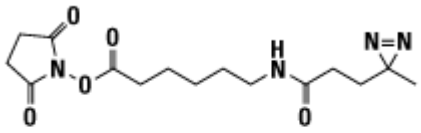
FT-DW8531

Diazirine crosslinkers
amine-photo-reactive

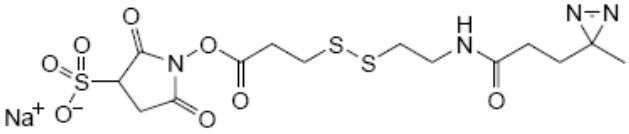
Products Description

membrane permeable for intracellular crosslinking (NHS Ester)	membrane impermeable for cell surface crosslinking (Sulfo-NHS Ester)
NHS-Diazirine(SDA) **DW8531, 50mg** Succinimidyl 4,4'-azipentanoate MW: 225.20 spacer: 3.9Å	**Sulfo-NHS-Diazirine (Sulfo-SDA)** **DW8561, 50mg** Sulfosuccinimidyl 4,4'-azipentanoate MW: 327.3 spacer: 3.9Å
LC-SDA (NHS-LC-Diazirine) **DW8541, 50mg** Succinimidyl 6-(4,4'-azipentanamido)hexanoate MW: 338.4 spacer: 12.5Å	**Sulfo-LC-SDA (Sulfo-NHS-LC-Diazirine)** **DW8571, 50mg** Sulfosuccinimidyl 6-(4,4'-azipentanamido)hexanoate MW: 440.4 spacer: 12.5Å
NHS-SS-Diazirine (SDAD) **DW8551, 50mg** Succinimidyl 2-([4,4'-azipentanamido]ethyl)-1,3'-dithioproprionate MW: 388.5 spacer: 13.6Å	**Sulfo-SDAD (Sulfo-NHS-SS-Diazirine)** **DW8581, 50mg** Sulfosuccinimidyl 2-([4,4'-azipentanamido]ethyl)-1,3'-dithioproprionate MW: 490.51 spacer: 13.6Å

NHS-LC-Diazirine:



sNHS-SS-Diazirine:



Storage: +4°C (L) – Protect from light and moisture

Succinimidyl-ester DiAzirine (SDA) reagents are a new class of crosslinkers that combine proven amine-reactive chemistry with an innovative and efficient **diazirine-based photochemistry** for conjugating amine-containing molecules to nearly any other functional group. The SDA crosslinkers include six compounds differing in spacer arm lengths, their ability to cleave the crosslinked proteins, and the presence or absence of a charged group for differential membrane permeability.

SDA reagents extend the efficiency and range of interactions that can be explored by standard protein crosslinking techniques used to understand **protein structure** and to stabilize **protein-protein interactions.**

Benefits :

- **Heterobifunctional** – succinimide ester group reacts with primary amines at pH 7-9 to form covalent amide bonds; diazirine group reacts efficiently with any amino acid side chain or peptide backbone upon activation with long-wave UV light (330-370 nm)
- **Controllable** – two-step chemical crosslinking is activated using common laboratory UV lamps
- **Easy to use** – these crosslinkers are photo-stable under typical laboratory lighting conditions so there is no need to perform experiments in the dark
- **Better than aryl azides** – the diazirine photo-reactive group has better photo-stabilityy in normal light than phenyl azide groups of traditional photo-reactive crosslinkers, yet the diazirine group is more efficiently activated by long-wave UV light
- **Versatile** – a variety of 6 different derivatives for specific experimental needs:
  Short (3.9 Å) and long (12.5 Å) spacer arms
  Membrane-permeable (NHS) and **impermeable** (Sulfo-NHS) analogs
  Non-cleavable and **cleavable** (disulfide spacer) derivatives

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Directions for use

Handling and Storage

All products are NHS esters, and sensitive to moisture. They should be kept at 4°C, and once opened if used partially, closed under inert dry gas (nitrogen, argon).

Dissolve NHS-ester diazirines stock solutions in dry DMSO or DMF. Dissolve Sulfo-NHS-ester diazirines in water or PBS. *See note [a]*.

Protocol 1 (r): In vitro Protein Crosslinking

The following example protocol should be adjusted for each application.

1. Prepare proteins in PBS at 54mg/ml. •*see note[a]*•
2. Prepare crosslinker solution immediately before use at 10 mM in suitable solvent. •*see note [b]*•
3. Add crosslinker to the protein sample at a final crosslinker concentration of 0.5-2 mM.

•*note*•If the protein concentration is ≥ 5 mg/ml, use a 10-fold molar excess of the crosslinker, or a 20- to 50-fold ratio for samples < 5 mg/ml.

4. Incubate the reaction at room temperature for 30 minutes or on ice for 2 hours.
5. Stop the reaction by adding quenching buffer (1 M Tris•HCl, pH 8.0) 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 suitable desalting tool (see related products).
8. Photoactivate diazirine-labeled proteins using UV lamp with 365 nm bulbs to photocrosslink proteins.•*see note [c]*•

Protocol 2 (r): Intra- and Extra-cellular Protein Crosslinking

Follow protocol 1, excepted sample preparation (step 1), and dessalting (step 7) that are performed by two washes with with ice cold PBS. Take car to cover cells completely with PBS during UV irradiation.

Cells should be analyzed directly or stored in suitable buffer.

Trouble shouting

Problem	Possible Cause	Solution
Minimal or no crosslinking	NHS-ester hydrolysis	Prepare new stock solution is dry using new dry DMSO or DMF (molecular sieve-treated, or commercial anhydrous grade).
		Add protein or cell sample immediately once diazirine reagent is dissolved in PBS –or even do no wait for complete solubilization-
	Inappropriate conjugation buffer	Check buffer does not contain primary amines such as Tris or glycine. •*See note[a]*•
	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
	Inappropriate photo-irradiation	Use UV wavelengths 320-370 nm for maximal photo-activation•*See note[c]*•
		Increase time of UV irradiation
		Decrease distance to UV bulbs•*See note[c]*•
Background, undesired conjugates	Excess crosslinker not quenched and/or removed	Use desalting/dialysis (or rinse cells thoroughly with PBS) before photo-crosslinking to remove non-derivatized crosslinker.
		Use multiple UV bulbs with > 8 W output and/or remove UV filters.
	Protein damaged or Cell death	Avoid using UV < 300 nm•*See note[c]*•

Technical and Scientific Information

- **Solubility and applications**

Non-sulfonated diazirine conjugates have no electric charge, and are membrane-permeable, which makes them useful for intracellular and intramembrane conjugations. Sulfonated diazirine conjugates are negatively charged (sulfate groups) and have reduced cell membrane permeability, making these crosslinkers useful for cell-surface protein crosslinking.

NHS-ester diazirine crosslinkers (SDA, LC-SDA and SDAD) are water-insoluble and may precipitate if added directly to aqueous buffer at concentrations > 5 mM: they should be dissolved in a dry water-miscible organic solvent, before adding to the aqueous reaction mixture.

Sulfo-NHS-ester diazirine derivatives are supplied as sodium salts, and water-soluble.

•*Note[a]*• Dissolve NHS-ester diazirine derivatives (SDA, LC-SDA, SDAD) at 10mM in a dry DMSO or DMF (i.e. 2mg pof LC-SDA in 591µl of DMSO), before diluting to < 10% solvent in final aqueous reaction buffer. The solubility decreases with increasing salt concentration.

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Sulfo-NHS-ester diazirine crosslinkers can be solubilized directly in water. they are also soluble in DMSO up to 10 mM.

- **Conjugation scheme**

The diazirine crosslinkers are typically used to conjugate 2 proteins. In a first step, the NHS group reacts with aminogroups of a first biomolecules; then the formed (diazirine-activated) conjugate will react as desired, upon photo-irradiation, with amino groups of an other biomolecule.

•*Note*• For best results, both hydrolyzed and non-reacted crosslinker are removed after reaction and quenching by suitable mean (gel filtration, dialysis).

For conjugation on suspended cells, culture media must be washed to remove amine containing substances. Higher diazirine concentration should be used on adherent cells which will be modified predominantly onto exposed surfaces.

- **NHS reactivity**

Succinimide ester (NHS) group reacts with primary amines at pH 7-9 to form covalent amide bonds. Acylation is favored near neutral pH (6-9) and with concentrated protein solutions, but competes with hydrolysis of the NHS ester. Other aminated substances in buffer should not be used!

•*Note[b]*• Use buffers at pH 7-9 such as PBS (100 mM sodium phosphate, 150 mM NaCl, pH 7.4) (#687213A); 20 mM HEPES; 100 mM carbonate/bicarbonate; or 50 mM borate. ***Do not use buffers that contain primary amines*** such as Tris or glycine which compete with acylation.

- **Diazirine reactivity**

Diazirine (azipentanoate) group reacts efficiently with any amino acid side chain or peptide backbone upon activation with long-wave UV light (330-370 nm).

•*Note* •NHS-Diazirine replace advantageously ANB-NOS #52219B (less efficient photo-reactive group).

•*Note[c]* •Use a **UV lamp** that irradiates between 320-370 nm, i.e. with 365nm bulb. High-wattage lamps are more effective and require shorter exposure times, while lower-wattage lamps(i.e. a hand-held 6 watt lamp) will result in lower crosslinking efficiency.

The optimal wavelength for diazirine photo-activation is 345 nm (Sukanek 2005). Filters that remove light at wavelengths below 300 nm are necessary for mercury vapor lamps. Do not use UV lamps that emit light at 254 nm as this wavelength causes proteins and DNA to photo-destruct.

•Samples **irradiation conditions** should be optimized. As guideline,
-for efficient UV irradiation, place the lamp above a shallow, uncovered reaction vessel, rotate the sample.

-the distance from lamp to samples may be 20cm for > 150watts lamps a 300 nm filter, or 3-5cm for a 15watt lamp, or 1cm for a 8watts lamp without filter.

-total UV irradiation time should be less than 15 minutes for crosslinking of live cells.

- **Spacer cleavage**

• NHS-SS-Diazirine (SDAD) and Sulfo-NHS-SS-Diazirine (Sulfo-SDAD) are useful when the crosslinked biomolecules should be disassembled in downstream applications. They disulfide bridge can be easily cleaved by reducers: 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.

References

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

Related / associated products and documents

See BioSciences Innovations catalogue and e-search tool.

*Other crosslinkers, i.e. other heterobifunctional crosslinkers with NHS and MAL reactivities:

• SANPAH #09649A (non iodinatable photo-reactive and amine reactive crosslinkers)
• MAL-PEOx-NHS #AL6580 (hydrophilic spacer), SMCC #17412A, GMBS #UP49608A & Sulfo-GMBS #, MBS #UP21608A, SMPB #UP28072A.
• Hydralink Chemistry: Conjugation kit #BL1501 and crosslinkers (SANH #BL9270, MHPH #BL9401).

*Associated products

• Phosphate-buffered saline (PBS) #UP687213A, TBS #UP09154D.
• Quenching Buffer (1 M Tris•HCl, pH 8.0) #
• Desalting Columns # and Dialysis devices or tubings #BB101a (i.e.Cellusep dialysis tubings)
• Protein Assays: Coo Assay #UPF86400, BC Assay UP40840A

Ordering information

Catalog size quantities and prices may be found at http://www.interchim.com.
Please inquire for higher quantities (availability, shipment conditions).

For any information, please ask : Uptima / Interchim; Hotline : +33(0)4 70 03 73 06

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