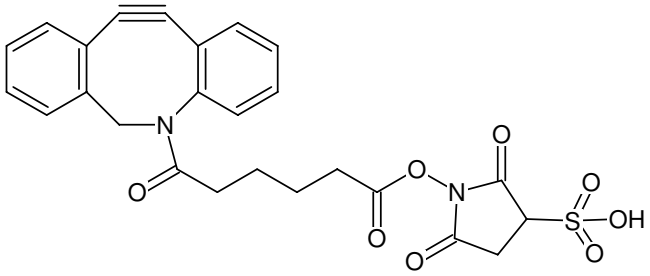
DATA SHEET

Jena Bioscience

DBCO-Sulfo-NHS ester (sodium salt)

Sulfo-Dibenzylcyclooctyne-NHS ester

Cat. No.	Amount
CLK-A124-10	10 mg
CLK-A124-100	10 x 10 mg



Structural formula of DBCO-Sulfo-NHS ester (sodium salt)

For research use only!

Shipping: shipped at ambient temperature

Storage Conditions: store at -20 °C

Shelf Life: 12 months after date of delivery

Molecular Formula: $C_{25}H_{21}N_2O_8S$ (free anion)

Molecular Weight: 509.51 g/mol (free anion)

CAS#: 1400191-52-7

Purity: > 90 % (HPLC)

Form: solid

Color: yellow to slightly grey

Solubility: DMF, DMSO, water

Applications:
Protein-peptide conjugates

Peptide-small molecule conjugates

^{18}F radiolabelling

Protein-oligonucleotide conjugates

Surface modification

Description:
The DBCO-sulfo-NHS ester is a water soluble, amine-reactive reagent for the incorporation of a DBCO moiety to an available amine functionality. Once the protein is DBCO-labeled, it can react with an azide-labeled molecule to produce a stable conjugate (triazole) via Cu(I)-free or strain-promoted click reaction. Because DBCOs and azides are absent from biological systems, there is minimal background labeling of cells or lysates.

Important Product Information

- NHS esters are moisture-sensitive. To avoid moisture condensation onto the product always let the vial come to room temperature before opening, be careful to limit exposure to moisture and restore under an inert atmosphere. The NHS-ester moiety readily hydrolyzes and becomes non-reactive, therefore, prepare stock solutions immediately before use. Stock solutions in anhydrous solvents can be kept for several days (freeze when not in use).
- Hydrolysis of the NHS ester is a competing reaction. Conjugation with primary amines of proteins/peptides (i.e. acylation) is favored at near neutral pH (6 - 9) and with concentrated protein solutions. For conjugation, use non-amine-containing buffers at pH 7 - 9 such as PBS (20 mM sodium phosphate, 150 mM sodium chloride, pH 7.4), 20 mM HEPES, 100 mM carbonate/biocarbonate, or 50 mM borate buffer.
- Do not use buffers that contain primary amines (e.g. Tris, Glycine).
- Avoid buffers that contain azides, which can react with DBCO.
- Dissolve DBCO-sulfo-NHS ester in water or in a dry water-miscible organic solvent such as DMSO or DMF before diluting in final reaction buffer. DBCO-sulfo-NHS ester is soluble in aqueous buffers.
- Reactions with DBCO and azides are more efficient at high concentrations and temperatures (i.e. 2 - 37 °C). Typical reaction times are less than 2 hours, however, incubating for longer can improve efficiency.

Additional Materials Required

- Water or water-miscible organic solvent such as dimethyl sulfoxide (DMSO) or dimethyl formamide (DMF)
- Reaction buffer: Phosphate-buffered saline (PBS) or other buf-

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fer at pH 5 - 9
- Quenching buffer: 1 M Tris·HCl, pH 8.0
- Spin Desalting Columns

Protein Derivitization
- Prepare proteins in PBS.
- Immediately before use, prepare 10 mM of the DBCO-sulfo-NHS reagent in water, DMSO or DMF.
- Add the NHS reagent to the protein sample at a final concentration of 0.5 - 2 mM. If the protein concentration is = 5 mg/ml, use a 10 - fold molar excess of the reagent. For samples < 5 mg/ml, use a 20 - to 50 - fold molar excess.
- Incubate the reaction at room temperature for 30 minutes or on ice for 2 hours.
- Stop the reaction by adding Quenching Buffer to a final concentration of 50 - 100 mM Tris.
- Incubate the reaction at room temperature for 5 minutes or on ice for 15 minutes.
- Remove non-reactive reagent by dialysis or desalting.

Copper-free Click Reaction
- Prepare the azide-containing sample in reaction buffer.
- Add DBCO-protein conjugate to azide-containing sample.
- Recommendation: Add 1 mol equivalent of limiting protein to 1.5 - 3.0 mol equivalents of highest abundance reagent.
- Incubate the reaction at room temperature for 2 - 4 hours or at 4 °C for 2 - 12 hours.
- The reaction is now ready for purification.

Long-term aqueous Stability of DBCO-labeled Samples

DBCO modified goat IgG (DOL 7) losses about 3 - 5 % of its reactivity toward azides over 4 weeks at 4 °C or -20 °C. For long time storage azide- and thiol-containing buffers should be avoided.

Direct Measurement of DBCO Incorporation

The degree of DBCO incorporation (i.e. the number of DBCO per protein molecule) can be determined from the absorbance scan of the purified conjugate (235 - 400 nm).

Troubleshooting

Problem: No conjugation of DBCO with azide
- Possible reason: One or more sample is not labeled
  - Confirm molecules were labeled or repeat activation process
- Possible reason: NHS ester hydrolyzed
  - Allow product to equilibrate to room temperature before opening
  - Prepare new solutions in the indicated dry solvents
  - Avoid buffers that contain primary amines such as Tris and glycine
- Possible reason: Excess reagent not quenched or removed
  - Remove excess NHS reagent by dialysis or desalting

Problem: Low conjugation of DBCO and azide
- Possible reason: Suboptimal reaction conditions
  - Increase incubation time
  - Optimize conjugation conditions by altering molar excess<br / >- Perform conjugation reactions at 37 °C

Selected References:

Simon *et al.* (2012) Facile Double-Functionalization of Designed Ankyrin Repeat Proteins using Click and Thiol Chemistries. *Bioconjugate Chem.* **23 (2)**:279.

Zeng *et al.* (2012). ^{64}Cu Core-Labeled Nanoparticles with High Specific Activity via Metal-Free Click Chemistry. *ACS Nano.* **6 (6)**:5209.

Arumugam *et al.* (2011). [^{18}F]Azadibenzocyclooctyne ([^{18}F]ADIBO): A biocompatible radioactive labeling synthon for peptides using catalyst free [3+2] cycloaddition. *Bioorg. Med. Chem. Lett.* **21**:6987.

Campbell-Verduyn *et al.* (2011). Strain-Promoted Copper-Free Click Chemistry for ^{18}F Radiolabeling of Bombesin. *Angew. Chem. Int. Ed.* **50**:11117.

Debets *et al.* (2010) Aza-dibenzocyclooctynes for fast and efficient enzyme PEGylation via copper-free (3+2) cycloaddition. *Chem. Commun.* **46**:97.