Click Chemistry Tools
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DBCO-PEG13-NHS Ester

Important Product Information

- NHS esters are moisture-sensitive. To avoid moisture condensation onto the product always let 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-PEG13-NHS ester in a dry water-miscible organic solvent such as DMSO or DMF before diluting in final reaction buffer. DBCO-PEG13-NHS ester is soluble in aqueous buffers up to 5.5 mM.
- Reactions with DBCO and azides are more efficient at high concentrations and temperatures (i.e., 2-37°C). Typical reaction times are less than 4 hours; however, incubating for longer can improve efficiency.

Procedure for Sample Labeling

Additional Materials Required

- Water-miscible organic solvent such as dimethyl sulfoxide (DMSO) or dimethyl formamide (DMF)
- Reaction buffer: Phosphate-buffered saline (PBS) or other buffer 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-PEG13-NHS reagent in 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

1. Prepare the azide-containing sample in reaction buffer.
2. Add DBCO-protein conjugate to azide-containing sample.

   Recommendation: Add 1 mole equivalent of limiting reagent to 1.5-3.0 mol equivalents of highest abundance reagent.
3. Incubate the reaction at room temperature for 4-12 hour. Incubation at 4^0C requires 2-12 hours.
4. The reaction is now ready for purification.

Troubleshooting

Problem	Possible Cause	Solution
No conjugation of DBCO with azide	One or more sample is not labeled	Confirm molecules were labeled or repeat activation process
	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
	Excess reagent not quenched or removed	Remove non-reacted reagent by dialysis or desalting
Low conjugation of DBCO and azide	Suboptimal reaction conditions	Increase incubation time
		Optimize conjugation conditions by altering molar excess
		Perform conjugation reactions at 37°C

References

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