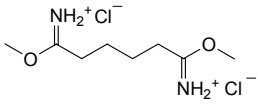
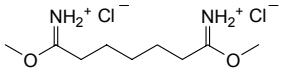
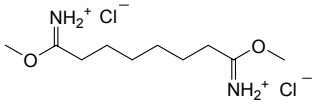
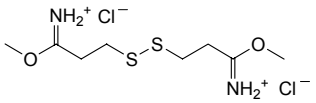
INSTRUCTIONS

Imidoester Crosslinkers: DMA, DMP, DMS, DTBP

0668.2

Number	Description	
20660	**DMA (Dimethyl adipimidate•2 HCl),** 1 g Molecular Weight: 245.15 Spacer Arm Length: 8.6 Å	
21667 **21666**	**DMP (Dimethyl pimelimidate•2 HCl),** 1 g **DMP (Dimethyl pimelimidate•2 HCl),** 50 mg Molecular Weight: 259.17 Spacer Arm Length: 9.2 Å	
20700	**DMS (Dimethyl suberimidate•2 HCl),** 1 g Molecular Weight: 273.20 Spacer Arm Length: 11.0 Å	
20665	**DTBP (Dimethyl 3,3'-dithiobispropio-nimidate•2HCl),** 1 g Molecular Weight: 309.28 Spacer Arm Length: 11.9 Å	

Storage: Upon receipt store dessicated at 4°C. Product is shipped at ambient temperature.

Introduction

DMA, DMP, DMS and DTBP are water soluble, membrane permeable, homobifunctional imidoester crosslinkers. The imidoester functional group is one of the most specific acylating groups available for the modification of primary amines and has minimal cross reactivity toward other nucleophilic groups in proteins.[1, 2] In addition, the imidoamide reaction product does not alter the overall charge of the protein, potentially retaining the native conformation and activity of the protein.

Important Product Information

- Imidoester crosslinkers are moisture sensitive. To avoid condensation onto the product, fully equilibrate vial to room temperature before opening (typically requires at least 30 minutes).
- For imidoester crosslinking reactions use buffers such as phosphate, borate, carbonate and HEPES that do not contain primary amines. Imidoesters react with amines at pH 7-10. For optimal crosslinking efficiency, use pH 8-9.
- Imidoester crosslinkers cannot be stored in solution because the imidate moiety is easily hydrolyzed.
- DMA, DMP and DMS are non-cleavable forms of imidoester crosslinkers. By contrast, crosslinks with DTBP can be cleaved by reducing the disulfide bond of the spacer arm with 100-150 mM DTT at 37°C for 30 minutes.

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General Procedure for Crosslinking Proteins

The following protocol is adapted from a procedure described by Packman and Perham.[3]

A. Materials Required

- Crosslinking Buffer: 0.2 M triethanolamine, pH 8.0. Do not use buffers that contain primary amines (e.g., Tris, glycine, etc.), as these buffers will compete with the crosslinking reaction.
- Stop Solution: Glacial acetic acid. Alternatively, Tris or glycine can be used to quench the reaction.

B. Procedure

1. Prepare the appropriate protein sample in crosslinking buffer.
2. Add a 10-fold molar excess of the cross-linker to the protein when the protein concentration is above 5 mg/ml. If the protein concentration is below 5 mg/ml add a 20- to 30-fold molar excess of the crosslinker.
3. Incubate the reaction at room temperature for 30-60 minutes.
4. Add glacial acetic acid at a 1:4 ratio to the sample to stop the reaction. Alternatively, stop the reaction by adding Tris or glycine at a 20-50 mM final concentration.

Related Thermo Scientific Products

20036	**Bioconjugate Techniques** (Book), 1202 pages, softcover
28384	**BupH™ Borate Buffer Packs,** 40 packs (each makes 500 ml of pH 8.5 buffer)
28372	**BupH Phosphate Buffered Saline Packs,** 40 packs (each makes 500 ml of pH 7.2 buffer)
20291	**DTT, No-Weigh™ Format,** 48 x 7.7 mg microtubes (each makes 500 mM solution upon addition of 100 µl of water)

References

1. Hand, E.S., and Jencks, W.P. (1962). Mechanism of the reaction of imidoesters with amines. *J. Am. Chem. Soc*. **84:** 3505-14.
2. Mattson, G., Conklin, E., Desai, S., Nielander, G., Savage, D., and Morgensen, S. (1993). A practical approach to crosslinking. *Mol. Biol. Rep*. **17:** 167-83.
3. Packman, L.C. and Perhan, R.N. (1982). Quaternary structures the pyruvate dehydrogenase multienzyme complex of *Bacillus stearothermophilus* studies by a new reversible crosslinking procedure with bis(imidoesters). *Biochem*. **21:** 5171-5.
4. Cole, N.B., *et al.* (2002). Lipid droplet binding and oligomerization of the Parkinson's disease protein alpha-synuclein. *J. Biol. Chem.* **277:** 6344-52.
5. Deleault, N., *et al.* (2005). Protease-resistant prion protein amplification reconstituted with partially purified substrates and synthetic polyanions. *J. Biol. Chem.* **280:** 26873-9.
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7. Mikhailov, V., *et al.* (2001). Bcl-2 prevents bax oligomerization in the mitochondrial outer membrane. *J. Biol. Chem.* **276:** 18361-74.