Introduction

DMA, DMP, DMS and DTBP (Figure 1) are water soluble, membrane permeable, homobifunctional imidoester cross-linkers. 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 cross-linkers are moisture sensitive. To avoid condensation onto the product, equilibrate vial to room temperature before opening (equilibration may require 30 minutes).

• For imidoester cross-linking reactions use buffers such as phosphate, borate, carbonate and HEPES that do not contain primary amines. For optimal results, cross-linking reactions should be performed at pH 7-9.

• Imidoester cross-linkers cannot be stored in solution because the imidate moiety is easily hydrolyzed.

• DMA, DMP and DMS are non-cleavable forms of imidoester cross-linkers. Conversely, the reaction with DTBP is reversible and can be cleaved with 100-150 mM DTT at 37°C for 30 minutes.

General Procedure for Cross-linking Proteins

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

Materials Required

• Cross-linking 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 cross-linking reaction.

• Stop Solution: Glacial acetic acid. Alternatively, Tris or glycine can be used to quench the reaction.
Procedure

1. Prepare the appropriate protein sample in cross-linking 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 20-30 fold molar excess of the cross-linker.
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.

![Molecular structure of DMA, DMP, DMS, and DTBP](image)

**Figure 1.** Molecular structure of DMA, DMP, DMS and DTBP

**Related Pierce Products**

- **20002** Bioconjugate Techniques, 785 pages, softcover
- **28372** BupH™ Phosphate Buffered Saline Packs, 40 packs
- **20290** DTT, 5 g

**References**