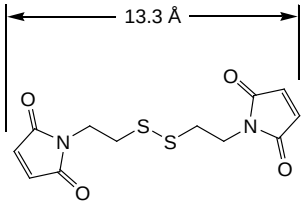
INSTRUCTIONS

# DTME

## 22335

0793.2

| Number | Description |
|---|---|
| **22335** | **DTME,** Dithio-bismaleimidoethane, 50mg<br>Molecular Weight: 312.37<br>Spacer Arm: 13.3Å |




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

## Introduction

Thermo Scientific DTME is a homobifunctional, maleimide crosslinker for conjugation between sulfhydryl groups (-SH). Such bismaleimide crosslinkers are commonly used to explore and characterize protein structure (i.e., oligomerization) or protein interactions. DTME is similar in length to BMH (Product No. 22330) but differs in containing a disulfide bond in its spacer arm, allowing crosslinks with DTME to be cleaved with reducing agent such as dithiothreitol (DTT).

Reaction of a sulfhydryl to the maleimide group results in formation of a stable thioether linkage (Figure 1), which cannot be cleaved by reducing agents or physiological buffer conditions. Reaction of maleimides is very specific to sulfhydryls at pH 6.5-7.5.[1] Although maleimides will react to primary amines at pH >8, the rate is 1000 times slower than the reaction to sulfhydryls at pH 7. Unlike iodoacetamides, maleimides do not react with tyrosines, histidines or methionines.

The maleimide moiety is temporarily stable in aqueous solutions devoid of reactive sulfhydryl targets, but hydrolysis to a nonreactive maleamic acid can occur during storage, especially at pH >8 (Figure 1). For this reason, dissolved reagents are best used promptly and the remainder discarded. Hydrolysis of the ring structure also can occur following conjugation, resulting in an open-ring linkage[2] (Figure 1).

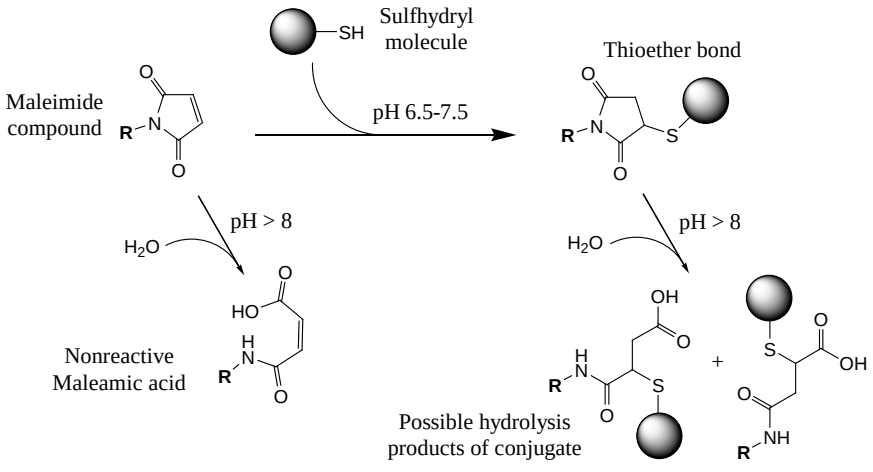



**Figure 1.** Reaction of maleimide-activated compounds to sulfhydryls.


Pierce Biotechnology 3747 N. Meridian Road | PO 117 Rockford, IL 61105 USA | (815) 968-0747 (815) 968-7316 fax | www.thermoscientific.com/pierce

## Important Product Information

- Molecules to be reacted with maleimide compounds must have free (reduced) sulfhydryls. Reduce peptide disulfide bonds with Thermo Scientific Immobilized TCEP Disulfide Reducing Gel (Product No. 77712). Reduce disulfide bonds in high molecular weight proteins using 5mM TCEP (1:100 dilution of Thermo Scientific Bond-Breaker TCEP Solution, Product No. 77720) for 30 minutes at room temperature, followed by TCEP removal using a desalting column (e.g., Thermo Scientific Zeba Spin Desalting Columns). Proteins (e.g., antibodies) can be inactivated by complete reduction of their disulfide bonds. Selective reduction of hinge-region disulfide bonds in IgG can be accomplished with 2-Mercaptoethylamine•HCl (2-MEA, Product No. 20408). Sulfhydryls can be added to molecules using *N*-succinimidyl *S*-acetylthioacetate (SATA, Product No. 26102) or 2-iminothiolane•HCl (Traut's Reagent, Product No. 26101), which modify primary amines.
- Avoid extraneous sulfhydryl-containing components in the reaction buffers during conjugation (e.g., DTT), as they react with the maleimide portion of the reagent, inhibiting and reducing conjugation efficiency of the intended target.
- The maleimide group reacts predominantly with free sulfhydryls at pH 6.5-7.5, forming stable thioether bonds. At pH values >7.5, reactivity toward primary amines and hydrolysis of the maleimide groups can occur. At pH 7, the maleimide group is ~1000 times more reactive toward a free sulfhydryl than to an amine.

## Procedure for Crosslinking Proteins in Solution

Generally, a two- or three-fold molar excess of crosslinker over the amount of sulfhydryl-containing protein(s) results in sufficient conjugation between proximal sulfhydryl groups. Empirical testing of reagent and protein concentrations is necessary to determine optimal conditions for the experiment.

### A. Material Preparation

- Conjugation Buffer: Phosphate buffered saline (PBS, pH 7.2; e.g., Product No. 28372) or other sulfhydryl-free buffer at pH 6.5-7.5. Include 5-10mM EDTA to help prevent the reoxidation of disulfides by trace divalent metals.
- Crosslinker Stock Solution: Immediately before use, weigh a small quantity of crosslinker and dissolve it in dimethylsulfoxide (DMSO) or dimethylformamide (DMF) at a 5-20mM concentration. For example, make a 20mM solution by dissolving 3.1mg DTME in 0.5mL DMSO or DMF.
- Sulfhydryl-containing protein, prepared as described the Important Product Information section.
- (Optional): Desalting column (e.g., Zeba™ Spin Desalting Columns) or dialysis unit (e.g., Thermo Scientific Slide-A-Lyzer Dialysis Cassettes) to separate crosslinked proteins from excess nonreacted crosslinker.

### B. Procedure for Protein Crosslinking

1. Dissolve protein(s) in Conjugation Buffer at 0.1mM (e.g., 5mg in 1mL for a 50kDa protein).
2. Add crosslinker to the dissolved protein(s) at 0.2mM final concentration (= two-fold molar excess for 0.1mM protein solution) by adding 10µL of Crosslinker Stock Solution per milliliter of protein solution.

   **Note:** The reaction solution may appear cloudy as a result of the low aqueous solubility of the crosslinker; usually, such solutions become clearer as the reaction proceeds. However, initial solubility can be increased by gentle heating and sonication. Other concentrations of Crosslinker Stock Solution can be used, as well as other final molar fold excesses of crosslinker. Most proteins remain soluble when the DMSO concentration does not exceed 10-15% of the final reaction volume; if protein solubility is not an issue, there is no limit to the DMSO concentration that may be used.
3. Incubate reaction mixture for 1 hour at room temperature or for 2 hours at 4°C.
4. (Optional) remove the excess nonreacted reagent by desalting or dialysis.

## Procedure for Cleaving DTME Crosslinks

Cleave DTME crosslinks (disulfide bonds) by treatment with 10-100mM reducing agent such as dithiothreitol (DTT, Product No. 20290, 20291) or TCEP (Product No. 20490, 77720). Heating for 5 minutes in typical reducing sample buffer for SDS-PAGE will also effectively reduce the crosslinks.

## Related Thermo Scientific Products

**Table 1. Bismaleimide Crosslinkers.**

| Crosslinker Name | Spacer Arm Length (Å) | Spacer Arm Composition (between maleimide groups) | Product No. |
|---|---|---|---|
| BMOE | 8.0 | Alkane | 22323 |
| BMDB | 10.2 | Cis-diol (periodate cleavable) | 22332 |
| BMB | 10.9 | Alkane | 22331 |
| BMH | 13.0 | Alkane | 22330 |
| DTME | 13.3 | Disulfide (reducing agent cleavable) | 22335 |
| $BM(PEO)_2$ | 14.7 | Polyethylene glycol (PEG) | 22336 |
| $BM(PEO)_3$ | 17.8 | Polyethylene glycol (PEG) | 22337 |

## Cited References


1. Smyth, D.G., Blumenfeld, O.O. and Konigsberg, W. (1964). Reaction of *N*-ethylmaleimide with peptides and amino acids. *Biochem J* **91:**589.
2. Partis, M.D., *et al.* (1983). Crosslinking of protein by w-maleimido alkanoyl *N*-hydroxysuccinimido esters. *J Protein Chem* **2(3):**263-277.


## Product References


Jessop, L., *et al.* (2000). The amino terminus of bacteriophage lambda integrase is involved in protein: protein interactions during recombination. *J Bacteriology* **182:**1024-34.

Kovalenko, O.V., *et al.* (2004). Evidence for specific tetraspanin homodimers: inhibition of palmitoylation makes cysteine residues available for crosslinking. *Biochem J* **344:**407-17.

Naithani, S., *et al.* (2003). Interactions among COX1, COX2 and COX3 mRNA-specific translational activator proteins on the inner surface of the mitochondrial inner membrane of Saccharomyces cerevisiae. *Mol Biol Cell* **14:**324-33.

Shmulevitz, M., Salsman, J. and Duncan, R.. (2003). Palmitoylation, membrane-proximal basic residues, and transmembrane glycine residues in the reovirus p10 protein are essential for syncytium formation. *J Virology* **77:**9769-79.