INSTRUCTIONS

2-Mercaptoethylamine•HCl

20408

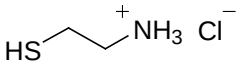
0131.3

Number	Description
20408	**2-Mercaptoethylamine•HCl (2-MEA)**, 6 × 6mg in amber screw-cap vials

Formula: C_2H_8ClNS

Molecular Weight: 113.61

CAS # 156-57-0



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

Introduction

2-Mercaptoethylamine•HCl (2-MEA) is a mild reducing agent suitable for selectively cleaving hinge-region disulfide bonds of IgG heavy chains. Several popular methods for conjugation or immobilization of antibodies involve covalent attachment specifically through sulfhydryl (−SH) groups. In IgG molecules, disulfide bonds between sulfur atoms in the side-chain of cysteine residues are important for maintaining IgG structure and function. Complete reduction of all disulfide bonds in an IgG generates individual heavy and light chains and obliterates its antigen-binding function. If disulfides only in the hinge region can be cleaved, however, two functional half-antibodies are produced with sulfhydryls available for crosslinking or immobilization methods (Figure 1).

Fortunately, 2-MEA is sufficiently mild to cleave disulfides between heavy chains of IgG or $F(ab)_2$ molecules while preserving the disulfide linkages between the heavy and light chains. This contrasts with other disulfide reducing agents, such as dithiothreitol (DTT) and 2-mercaptoethanol (2-ME) that will cleave all disulfide bonds in IgG molecules at nearly equal rates. Therefore, for most circumstances, only 2-MEA is suitable for selective, partial reduction of immunoglobulins.

EDTA is an important component in the IgG reduction reaction. By chelating divalent metal cations, EDTA prevents oxidation, helping to maintain sulfhydryls in a reduced form. Sulfhydryl groups generated in the IgG hinge region are fairly stable in the presence of EDTA. In one study, the number of sulfhydryls in reduced IgG and Fab' decreased by only 7 and 9%, respectively, in the presence of EDTA after 40 hours but decreased 63-90% and 15-25% in the absence of EDTA.[1]

Figure 1. Selective reduction of hinge region disulfide bonds in IgG with 2-MEA.

Procedure for Selective Reduction of Hinge-Region Disulfides in IgG

The following method may be modified for reducing proteins, peptides or other molecules besides IgG. Generally, the protein/peptide concentration is not as critical as the absolute concentration of 2-MEA and the presence of EDTA. Incubation time and other parameters must be optimized empirically to achieve the desired partial disulfide reduction.

Note: Results may vary depending on the specific IgG and the number of disulfide bonds within the hinge region.

A. Additional Materials Required

- Reaction Buffer: Phosphate-buffered saline containing EDTA (PBS-EDTA): 20-100mM sodium phosphate, 150mM NaCl, 1-10mM EDTA, pH 7.2. Other buffers at other pH values may be used but must include 1-10mM EDTA.
- IgG dissolved at 10mg/mL in Reaction Buffer or other compatible buffer containing 1-10mM EDTA
- Incubator or water bath equilibrated to 37°C
- Desalting columns sufficient to process the sample volume used (see Related Thermo Scientific Products)

B. Procedure

1. Add 1mL of IgG solution to one vial that contains 6mg of 2-Mercaptoethylamine•HCl (results in 50mM 2-MEA).

   Note: If the starting IgG sample is less than 1mL, first dissolve the 6mg of 2-MEA in 100µL Reaction Buffer and then immediately add 1µL of this 2-MEA stock to each 10µL volume of sample.

2. Cap, mix well to dissolve the 2-MEA, and incubate reaction mixture for 90 minutes at 37°C.
3. Cool reaction to room temperature and separate the 2-MEA from the reduced IgG using a desalting column. Pre-equilibrate the desalting column with Reaction Buffer (PBS-EDTA) and use this same buffer for buffer exchange.
4. Perform a buffer exchange according to the instructions for the desalting column.

   Note: Make sure that the amount of 2-MEA removed is sufficient for using the reduced IgG in reactions with thiol-reactive reagents. Any remaining reductant should be at levels far below the mole amount of IgG or it will be a significant competitor in subsequent reactions.

Related Thermo Scientific Products

89891	**Zeba™ Spin Desalting Columns, 5mL,** 5 each, for 500-2000µL samples
89892	**Zeba Spin Desalting Columns, 5mL,** 25 each, for 500-2000µL samples
44895	**SulfoLink® Kit**, for covalent immobilization of molecules through sulfhydryls to agarose resin
77712	**Immobilized TCEP Disulfide Reducing Gel**
21920, 21930	**EZ-Link® Maleimide-PEG Solid Phase Biotinylation Kits**

Cited Reference

1. Yoshitake, S., *et al.* (1979). Conjugation of glucose oxidase from *Aspergillus niger* and rabbit antibodies using *N*-hydroxysuccinimide ester of N-(4-carboxycyclohexyl-methyl)-maleimide. *Eur J Biochem* **101:**395-9.

Product References

Cefai, D., *et al.* (1998). CD28 Receptor Endocytosis is targeted by mutations that disrupt phosphatidylinositol 3-kinase binding and costimulation. *J Immunol* **160:**2223-30.

Epstein, A.L., *et al.* (2003). Identification of a protein fragment of interleukin 2 responsible for vasopermeability. *J Nat Cancer Inst* **95(10):**741-9.

Mahnke, K. *et al*. (2003). Induction of CD4+/CD25+ regulatory T cells by targeting of antigens to immature dendritic cells. *Blood* **101:**4862-9.

Stimmel, J.B., *et al.* (2000). Site-specific conjugation on serine-cysteine variant monoclonal antibodies. *J Biol Chem* **275(39):**30445-50.