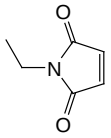
INSTRUCTIONS

N-Ethylmaleimide (NEM)

23030 1779.1

Number	Description
23030	***N*-Ethylmaleimide(NEM),** 25g Molecular Weight: 125.13



Storage: Upon receipt store at -20°C. Product shipped on ice pack.

Introduction

Thermo Scientific™ *N*-Ethylmaleimide (NEM) is an alkylating reagent that reacts with sulfhydryls to form stable thioether bonds.[1] At pH 6.5-7.5, the maleimide reaction is specific for sulfhdryls.[1-4] At pH values > 7.5, reactivity with amino groups occurs.[5] Maleimide groups react with sulfhydryls by nucleophilic attack of the thiolate anion on one of the carbons of the double bond. When sufficient sulfhydryls have been blocked, the reaction can be monitored by the decrease in absorbance at 300nm as the double bond becomes a single bond. The resulting thioether group is non-reversible and terminates in an ethyl group, blocking or capping the sulfhydryl.[6]

Procedure or Protocol for Blocking Sulfhydryls on a Protein

Note: Maleimides react with sulfhydryls at pH 6.5-7.5 to form stable thioether bonds. At pH values > 7.5, reactivity toward primary amines and hydrolysis of the maleimide group can occur.

A. Material Required

- Reaction Buffer: Phosphate-buffered saline (PBS) containing 0.1M phosphate, 0.15M sodium chloride; pH 7.2 (Thermo Scientific™ BupH™ Phosphate Buffered Saline Packs, Product No. 28372) or other amine-free buffer at pH 6.5-7.5
- Device to remove excess NEM, such as Thermo Scientific™ Slide-A-Lyzer™ Dialysis Cassettes, Slide-A-Lyzer MINI Dialysis Units or Zeba™ Spin Desalting Columns (see the Related Thermo Scientific Products Section)

B. Blocking of Sulfhydryls on Proteins

1. Equilibrate the NEM to room temperature before opening to prevent condensation in the bottle and subsequent hydrolysis and loss of function.
2. Dissolve protein to be blocked in Reaction Buffer at 1-10mg/mL.
3. Prepare 100-200mM of NEM in ultrapure water. Add a minimum of a 10-fold molar excess of NEM to sulfhydryl groups to be blocked. Alternatively, add an equal mass amount of NEM to the protein (e.g., add 2mg of NEM to 1mL of 2mg/mL protein).

   Note: Prepare the NEM solution immediately before use to prevent hydrolysis of the maleimide group.
4. React for 2 hours at room temperature.
5. Dialyze or desalt the blocked protein to remove excess NEM.

   Note: Ellman's Reagent (Product No. 22582) can be used to quantify the level of remaining sulfhydryls after the reaction with NEM.

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Troubleshooting

Problem	Possible Cause	Solution
Sulfhydryls not blocked	Hydrolysis of reagent	Equilibrate vial to room temperature before opening and minimize exposure to air; for best results, purge air from vial using a gentle stream of nitrogen gas
		Make NEM solutions immediately before each use and dispose of excess reconstituted reagent
Sulfhydryls partially blocked	Insufficient NEM used	Use at least a 10-fold excess of NEM to sulfhydryls
	Incorrect reaction buffer	Avoid buffers that contain sulfhydryls or amines, as they can compete with the intended reaction
	Insufficient reaction time	Allow reaction to proceed for at least 2 hours at room temperature or 4-12 hours at 4°C
Amines labeled with NEM	Reaction Buffer pH was greater than 7.5	Maintain the reaction buffer pH at 6.5-7.5

Related Thermo Scientific Products

69576 **Slide-A-Lyzer MINI Dialysis Unit Kit**

66382 **Slide-A-Lyzer Dialysis Cassette Kits**

89882 **Zeba Desalt Spin Columns, 7K MWCO, 0.5mL**

89889 **Zeba Desalt Spin Columns, 7K MWCO, 2mL**

22582 **Ellman's Reagent**, 5g

28372 **BupH Phosphate Buffered Saline Packs**, 40 packs

Cited References

1. Smyth, D.G., *et al.* (1960). Reactions of *N*-ethylmaleimide. *J Am Chem Soc* **82:**4600-4.
2. Heitz, J.R., *et al.* (1968). Inactivation of yeast alcohol dehydrogenase by *N*-alkylmaleimides. *Arch Biochem Biophys* **127:**627-8.
3. Gorin, G., *et al.* (1966). Kinetics of the reaction of *N*-ethylmaleimide with cysteine and some congeners. *Arch Biochem Biophys* **115:**593-7.
4. Partis, M.D., *et al*. (1983). Cross-linking of protein by ω-maleimido alkanoyl *N*-hydroxysuccinimido esters. *J Protein Chem* **2:**263-77.
5. Brewer, C.F. and Riehmn, J.P. (1967). Evidence for possible nonspecific reactions between *N*-ethymaleimide and proteins. *Anal Biochem* **18:**248-55.
6. Haugaard, N., *et al.* (1981). Use of *N*-ethylmaleimide to prevent interference by sulfhydryl reagents with the glucose oxidase assay for glucose. *Anal Biochem* **116:**341-3.

Product Reference

Hajra, A.K., *et al.* (2000). Induction of the peroxisomal glycerolipid-synthesizing enzymes during differentiation of 3T3-L1 adipocytes. *J Biol Chem* **275(13):** 9441-6.