INSTRUCTIONS

TNBSA
(2,4,6-Trinitrobenzene Sulfonic Acid)

TS-28997 0864w

Product Description

Number	Description
TS-28997	**TNBSA** (2,4,6-Trinitrobenzene Sulfonic Acid); 5% w/v in methanol, *100 ml*

Introduction

2,4,6-Trinitrobenzene Sulfonic Acid (TNBSA or TNBS) is a rapid and sensitive assay reagent for the determination of free amino groups.[1] Primary amines, upon reaction with TNBSA, form a highly chromogenic derivitive, which can be measured at 335 nm (see figure). Qualitative measurements of amines, sulfhydryls or hydrazides,[3] and quantitative measurements of ∑-amino groups of L-lysine[4] have also been obtained using TNBSA.

Example Protocol

The following protocol is adapted from a procedure described by Hermanson.[4]

Materials Required

Reaction Buffer: 0.1 M sodium bicarbonate, pH 8.5

TNBSA: 0.01% (w/v) solution of TNBSA. Prepare using reaction buffer as a diluent. Prepare fresh for each reaction.

10% solution of SDS in distilled water

1 N HCl

Method

1. Dissolve proteins to be assayed directly in reaction buffer at a concentration of 20-200 µg/ml. Alternatively, for proteins already in solution, the buffer can be changed by dialysis. Small molecules such as amino acids should be dissolved in reaction buffer at a concentration of 2-20 µg/ml.
   Caution: Tris, glycine or other buffers containing free amines should be avoided because the free amines will react with TNBSA.
2. Add 0.25 ml of the 0.01% (w/v) solution of TNBSA to 0.5 ml of each sample solution. Mix well.
3. Incubate at 37°C for two hours.
4. Add 0.25 ml of 10% SDS and 0.125 ml of 1 N HCl to each sample.
5. Measure the absorbance of the solutions at 335 nm.

Quantitative determination of the number of amines contained within a sample can be accomplished through comparison to a standard curve generated by the use of an amine-containing compound (*e.g.*, amino acid) dissolved in a series of known concentrations. The standards should be dissolved or dialyzed into the reaction buffer and must be assayed under reaction conditions identical to those utilized for the samples.

References

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5. Endo, N., Umemoto, N., Kato, Y., Takeda, Y. and Hara, H. (1987). A novel covalent modification of antibodies at their amino groups with retention of antigen-binding activity. *J. Immunol.* **104**, 253-258.
6. Sashidhar, R.B., Capoor, A.K. and Ramana, D. (1994). Quantitation of ∑-amino group using amino acids as reference standards by trinitrobenzene sulfonic acid. *J. Immunol.* **167**, 121-127.
7. Morcol, T., Subramanian, A. and Velander, W. (1997). Dot-blot analysis of the degree of covalent modification of proteins and antibodies at amino groups. *J. Immunol.* **203**, 45-53.
8. Bubnis, W. and Ofner, C. (1992). The determination of epsilon-amino groups in soluble and poorly soluble proteinaceous materials by a spectrophotometric method using trinitrobenzenesulfonic acid. *Anal Biochem.* **297**, 129-33.

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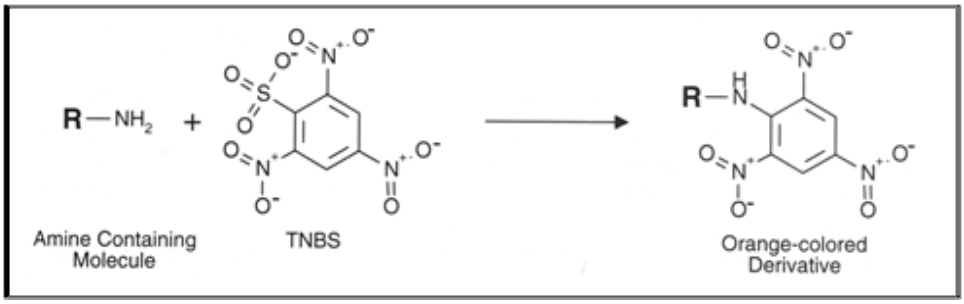

Reaction of TNBSA with a primary amine-containing molecule to produce a chromogenic derivative.