# **INSTRUCTIONS**

# TNBSA



0864.2

28997

Number

Description

28997

Storage: Upon receipt store at-20°C. Product is shipped at ambient temperature.

# Introduction

Thermo Scientific TNBSA (2,4,6-trinitrobenzene sulfonic acid) is a rapid and sensitive reagent for quantitating free amino groups. TNBSA reacts with primary amines to form a highly chromogenic derivative, which can be measured at 335nm. TNBSA can be used for measuring amines, sulfhydryls, hydrazides, and  $\varepsilon$ -amino groups of L-lysine.

# **Example Protocol for Quantitating Amino Groups**

## A. Materials Required

- Reaction Buffer: 0.1M sodium bicarbonate, pH 8.5
- 10% sodium dodecyl sulfate (SDS) in water
- 1N HCl

## B. Method

**Note:** To quantitate amines in a sample, compare results to a standard curve generated with an amine-containing compound (e.g., amino acid) dissolved in a series of known concentrations. Dissolve or dialyze the standards into the Reaction Buffer and assay using the same reaction conditions as the unknown samples.

- 1. Immediately before use prepare 0.01% (w/v) TNBSA using Reaction Buffer as the diluent.
- 2. Dissolve proteins directly in Reaction Buffer at 20-200µg/mL. Alternatively, for proteins already in solution, the buffer can be changed by dialysis. Dissolve small molecules, such as amino acids, in reaction buffer at 2-20µg/mL.

Caution: Avoid Tris, glycine or other buffers containing free amines.

- 3. Add 0.25mL of the 0.01% TNBSA to 0.5mL of each sample and mix well.
- 4. Incubate at 37°C for 2 hours.
- 5. Add 0.25mL of 10% SDS and 0.125mL of 1N HCl to each sample.
- 6. Measure the absorbance at 335nm.



#### **General References**

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Inman, J.K. and Dintzis, H.M. (1969). The derivatization of cross-linked polyacrylamide beads. Controlled introduction of functional groups for the preparation of special-purpose, biochemical adsorbents. *Biochem* 8:4074-82.

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