2,2'-AZINO-BIS-(3-ETHYLBENZTHIAZOLINE-6-SULFONIC ACID)

ABTS™

PRODUCT NO. ABTS

For Kinetic or Endpoint Assays of Horseradish Peroxidase Labeled Probes

INTRODUCTION

ABTS is considered a safe-sensitive substrate for horseradish peroxidase (HRP) based ELISA assays. In the presence of hydrogen peroxide and HRP, ABTS is oxidized to a radical cation showing adsorption maxima at 820 nm, 734 nm, 650 nm and 405 nm. The latter frequency demonstrates a significant molar extinction coefficient and is generally employed for most ABTS assays. Stopping the reaction with acid does not alter the 405 nm spectrum allowing kinetic and endpoint methods to be measured at the same wavelength. Using a proprietary-nontoxic stabilization technology, **MOSS, INC**. provides a room temperature stable, single component ABTS solution satisfactory for quantitative analysis of HRP based systems.

METHOD SYNOPSIS

After completion of analyte binding to a solid phase and reaction with an HRP labeled probe, ABTS Solution is added. Oxidation of ABTS produces a blue-green reaction product that is measured at 405-410 nm. The color formation as a function of time can be recorded or the reaction may be stopped by addition of acid.

REAGENT PROVIDED

ABTS SOLUTION: Contains ABTS, 1.46 mMol L^{-1}, in a citrate buffer, pH 4.0. Also contains stabilizers.

May be stored at room temperature, 15-28 ^{o}C. Refrigerator temperatures, 2-8 ^{o}C, will not harm the product. It must be warmed to assay temperature prior to use.

Discard if solution becomes bright yellow or turbid.

PROTECT FROM EXPOSURE TO DIRECT SUNLIGHT.

REAGENTS REQUIRED TO STOP THE REACTION, BUT NOT PROVIDED BY MOSS, INC.

STOPPING SOLUTION: A 0.625 Mol L^{-1} Oxalic Acid solution is recommended. Other acids may be employed.

PROCEDURE

1. Complete all required incubations with antibodies and HR labeled probes.

2. Wash plate wells at least 4 times with phosphate-buffered saline or tris-buffered saline containing 0.1% Tween-20.

3. After the final wash, shake and blot all residual buffer from the wells.

4. Add 100 microliters of ABTS Solution to each well and incubate at room temperature for 30 minutes. Readings at 405 nm can be taken at predetermined intervals if a kinetic assay is desired.

5. After 30 minutes, add 100 microliters of stop solution, mix well and read absorbance at 405 nm. The color is stable for at least 1 hour if 0.625 Mol L^{-1} oxalic acid is used.

The method described is similar to that employed by **MOSS, INC.** Variations of time, reagent volumes and temperature require further standardization by the user.

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