ABTS™

For Kinetic or Endpoint Assays of Horseradish Peroxidase Labeled Probes

rev.10/10

For research use only

ALTERNATE NAME: 2,2'-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)

CATALOG #: 1212-100

AMOUNT: 100 ml

STORAGE CONDITIONS: 2-8˚C

SHELF LIFE: Stable for up to 12 months at -20˚C

ABTS SOLUTION: Contains ABTS, 1.46 mMol L^{-1}, in a citrate buffer, pH 4.0. Also contains stabilizers. *Warm to assay temperature before use.*

INTRODUCTION:

ABTS is considered a safe and sensitive substrate compatible for horseradish peroxidase (HRP) based ELISA assays. In the presence of hydrogen peroxide and HRP, ABTS is oxidized to a radical cation with an 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, BioVision, Inc. provides a room temperature stable, single component ABTS solution for quantitative analysis of HRP based systems.

ASSAY DESCRIPTION:

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.

STOP SOLUTION: A 0.625 Mol L^{-1} Oxalic Acid solution is recommended. Other acids may be employed. *(Not provided).*

PROTOCOL:

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 buffers from the wells.
4. Add 100 µl 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 µl 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.

NOTE: Protect from direct sunlight. Discard if solution is blue or turbid.

FOR RESEARCH USE ONLY! Not to be used in humans.

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