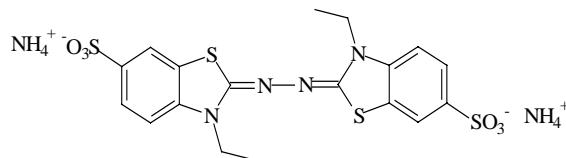


**2,2'-AZINO-BIS(3-ETHYLBENZTHIAZOLINE-6-SULFONIC ACID)
DIAMMONIUM SALT**
Sigma Prod. No. A1888

CAS NUMBER: 30931-67-0
SYNONYM: ABTS¹

PHYSICAL DESCRIPTION:

Appearance: powder, light yellow-green to green²
Molecular formula: C₁₈H₁₈N₄O₆S₄·(NH₃)₂
Molecular weight: 548.7
E_{MM}(342 nM) ≥ 37.4
E_{MM}(225 nM) ≥ 48.15³
E_{MM}(414 nM) = 36⁴



ABTS has been used as a chromogenic substrate for horseradish peroxidase (HRP), both in general activity assays and in ELISA applications. Activity of HRP using ABTS appears about four-fold higher than using pyrogallol.² Sigma's protocol is a continuous spectrophotometric assay.^{2,9,11}

The choice of substrate for peroxidase depends on application: sensitivity needed, solubility or color requirements for the product, stability of the substrate and comparative hazards for the user (long-term or short-term). A number of studies address these criteria.^{7,12-14} Detailed kinetic data and analyses have been reported.⁴

The peroxidase-catalyzed reduction of hydrogen peroxide to water is coupled to a one-electron oxidation of ABTS, forming a metastable radical cation.⁴ This cation gives a brilliant blue-green solution, $\lambda_{\text{max}} = 414$ nm and additional peaks at 395, 640 and 725 nm. Reduced ABTS has a characteristic peak at 340 nm; the isosbestic point is approximately 375 nm.⁵

The protocol for using ABTS with Peroxidase P6782 is available on request or through the Sigma World Wide Web site.

STABILITY / STORAGE AS SUPPLIED:

2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS¹) has a shelf-life of three years. The compound is considered light-sensitive.²

SOLUBILITY / SOLUTION STABILITY:

ABTS dissolves at 50 mg/mL water to give a clear to very slightly hazy solution that is light yellow green to green in color. Reduced ABTS is colorless, whereas oxidized ABTS is dark green in solution.^{5,6} ABTS solutions are sensitive to oxidation, particularly in the presence of heavy metal ions. Solutions should be prepared fresh each day, stored on ice until use. Under these conditions, the solution shows essentially no change in absorbance in ten hours.⁹ A solution at room temperature exposed to oxygen and light may turn dark green within an hour or two.¹⁰ (Sigma has ABTS in tablet form for convenient use.)

General usage is in a buffer compatible with peroxidase: acetate, phosphate or phosphate-citrate buffer at 50-100 mM, pH 5.0. A study of different buffers strongly recommends avoiding any of the "Good buffers", for example, MES or TES; they catalyze the reduction of the colored ABTS radical cation back to the reduced form.⁵ ABTS may be used at different concentrations (from 0.02 mM to 9.1 mM) in phosphate buffer (25 mM to 100 mM) and pH 5 to pH 7.^{4,9,15} Sigma protocols suggest 10 mg in 100 mL of 50 mM phosphate-citrate buffer pH 5.0 (about 0.2 mM)¹⁰, or at 9.1 mM in 100 mM phosphate buffer (as noted in Sigma's HRP assay).

Solution stability may be improved by dissolving the ABTS in deaerated buffer, then storing the solution under argon or nitrogen in aliquots at -20°C.¹¹

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