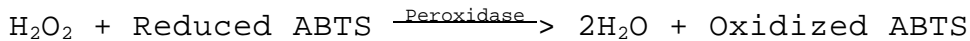


Enzymatic Assay of LACTOPEROXIDASE¹
(EC 1.11.1.7)
2,2'-Azino-bis(3-Ethylbenzthiazoline-6-Sulfonic Acid)
as a Substrate

PRINCIPLE:



Abbreviation used:

ABTS² = 2,2'-Azino-bis(3-Ethylbenzthiazoline-6-Sulfonic Acid)

CONDITIONS: T = 25°C, pH = 5.5, A_{436nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 100 mM Potassium Phosphate, Monobasic Solution
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379.)
- B. 100 mM Potassium Phosphate Dibasic Solution
(Prepare 100 ml in deionized water using Potassium Phosphate, Dibasic, Trihydrate, Sigma Prod. No. P-5504.)
- C. 100 mM Potassium Phosphate buffer, pH 5.5 at 25°C
(Prepare 100 ml by adjusting the pH of Reagent A to 5.5 at 25°C with Reagent B.)
- D. 100 mM 2,2'-Azino-bis(3-Ethylbenzthiazoline-6-Sulfonic Acid) Substrate Solution (ABTS)²
(Prepare 30 ml in deionized water using 2,2'-Azino-bis(3-Ethylbenzthiazoline-6-Sulfonic Acid), Diammonium Salt, Sigma Prod. No. A-1888. **PREPARE FRESH.**)
- E. 0.025% (w/w) Hydrogen Peroxide Solution (H₂O₂)
(Prepare 50 ml in cold deionized water using Hydrogen Peroxide, 30% (w/w) Solution, Sigma Prod. No. H-1009. **PREPARE FRESH.**)³

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REAGENTS: (continued)

- F. 200 mM Potassium Phosphate Solution (Enz Dil)
 (Prepare 100 ml in deionized water using Potassium Phosphate, Dibasic, Trihydrate Sigma Prod. No. P-5504.)
- G. Lactoperoxidase Enzyme Solution
 (Immediately before use, prepare a solution containing 0.15 - 0.25 unit/ml of Peroxidase in cold Reagent F.)

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable cuvettes:

	<u>Test</u>	<u>Blank</u>
Reagent C (Buffer)	2.20	2.20
Reagent D (ABTS)	0.70	0.70
Reagent E (H ₂ O ₂)	0.10	0.10

Mix by inversion and equilibrate to 25°C. Monitor the A_{436nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent G (Enzyme Solution)	0.05	-----
Reagent F (Enz Dil)	-----	0.05

Immediately mix by inversion and record⁴ the increase in A_{436nm} for approximately 2 minutes.⁵ Obtain the r A_{436nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\text{r } A_{436\text{nm}}/\text{min Test} - \text{r } A_{436\text{nm}}/\text{min Blank})(3.05)(\text{df})}{(29.3) (0.05)}$$

- 3.05 = Total volume (in milliliters) of assay
- df = Dilution factor
- 29.3 = Millimolar extinction coefficient⁶ of oxidized ABTS at 436nm
- 0.05 = Volume (in milliliter) of enzyme used

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CALCULATIONS: (continued)

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/mg enzyme}}$$

UNIT DEFINITION:

One unit will oxidize 1.0 μ mole of 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) per minute at pH 5.5 at 25°C.

FINAL ASSAY CONCENTRATIONS:

In a 3.05 ml reaction mix, the final concentrations are 75 mM potassium phosphate, 23 mM 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid), 0.0008% (w/w) hydrogen peroxide, and 0.0075 - 0.0125 unit peroxidase.

REFERENCE:

Keesey, J. (1987) in *Biochemica Information*, pp. 49, First Edition, Boehringer Mannheim Biochemicals, Indianapolis, IN

Pütter, J. and Becker, R. (1983) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U., ed.) 3rd ed., Vol III, pp. 286-293, Verlag Chemie, Deerfield Beach, FL

NOTES:

1. This assay is to be used to assay the activity of Lactoperoxidase from Bovine Milk, Sigma Prod. Nos. L-8257, L-2005, L-2130, and L-0515. This assay is also used to determine the suitability of 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid), Diammonium Salt, Sigma Prod. No. A-1888 as a substrate for lactoperoxidase.
2. ABTS is a registered trademark of Boehringer Mannheim GmbH.
3. The absorbance of this solution should be

approximately 0.4 measured at $A_{240\text{nm}}$.

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NOTES: (continued)

4. The rate should be measured at approximately 120 readings/minute.
5. The maximum linear rate occurs within the first minute of the reaction.
6. The millimolar extinction coefficient is cited in Keesey, J. (1987).
7. This assay is based on the cited references.
8. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.