SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of PEROXIDASE (EC 1.11.1.7) 2,2'-Azino-bis(3-Ethylbenzthiazoline-6-Sulfonic Acid) as a Substrate Sigma Prod. No. P-6782

PRINCIPLE:

H_2O_2 + ABTS $\xrightarrow{\text{Peroxidase}}$ > $2H_2O$ + oxidized ABTS

Abbreviation used:
ABTS[1] = 2,2'-Azino-bis(3-Ethylbenzthiazoline-6-Sulfonic Acid)

CONDITIONS: T = 25°C, pH = 5.0, A_{405nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Potassium Phosphate Buffer, pH 5.0 at 25°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Sigma Prod. No. P-5379. Adjust to pH 5.0 at 25°C using 1.0 M KOH.)

B. 9.1 mM 2,2'-Azino-bis(3-Ethylbenzthiazoline-6-Sulfonic Acid) Substrate Solution (ABTS[1])
(Prepare 30 ml in Reagent A using 2,2'-Azino-bis(3-Ethylbenzthiazoline-6-Sulfonic Acid), Tablets Diammonium Salt, Sigma Prod. No. A-9941. **PREPARE FRESH.**)

C. 0.3% (w/w) Hydrogen Peroxide Solution (H_2O_2)
(Prepare 50 ml in deionized water using Hydrogen Peroxide, 30% (w/w) Solution, Sigma Prod. No. H-1009. **PREPARE FRESH**.)

D. 40 mM Potassium Phosphate Buffer with 0.25% (w/v) Bovine Serum Albumin and 0.5% (v/v) Triton X-100[2], pH 6.8 at 25°C (Enzyme Diluent)
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Sigma Prod. No. P-5379, Albumin, Bovine, Sigma Prod. No. A-4503, Triton X-100, Sigma Stock No. X-100. Adjust to pH 6.8 at 25°C using 1 M K0H.)

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REAGENTS: (continued)

E. Peroxidase Enzyme Solution
(Prepare an enzyme stock solution containing 10 mg/ml in cold Reagent D. Immediately before use, prepare a solution containing 0.20 - 0.80 unit/ml of Peroxidase in cold Reagent D.)

PROCEDURE:

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

	Test	Blank
Reagent B (ABTS)	2.90	2.90
Reagent D (Enzyme Diluent)	------	0.05
Reagent E (Enzyme Solution)	0.05	------

Mix by inversion and equilibrate to 25°C. Monitor the A_{405nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent C (H_2O_2)	0.10	0.10

Immediately mix by inversion and record the increase in A_{405nm} for approximately 2 minutes.[3] Obtain the ΔA_{405nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{405nm}/\text{min Test} - \Delta A_{405nm}/\text{min Blank})(3.05)(df)}{(36.8)\,(0.05)}$$

3.05 = Total volume (in milliliters) of assay
df = Dilution factor
36.8 = Millimolar extinction coefficient of oxidized ABTS at 405nm
0.05 = Volume (in milliliter) of enzyme used

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

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CALCULATIONS: (continued)

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

UNIT DEFINITION:

One unit will oxidize 1.0 µmole of 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) per minute at pH 5.0 at 25°C.

FINAL ASSAY CONCENTRATIONS:

In a 3.05 ml reaction mix, the final concentrations are 96 mM potassium phosphate, 8.7 mM 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid), 0.01% (w/w) hydrogen peroxide, 0.004% (w/v) bovine serum albumin, 0.008% (v/v) Triton X-100 and 0.01 - 0.04 unit peroxidase.

REFERENCE:

Keesey, J. (1987) in *Biochemica Information,* pp. 58, 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, Verlug Chemie, Deerfield Beach, FL

NOTES:

1. ABTS is a registered trademark of Boehringer Mannheim GmbH.
2. Triton is a registered trademark of the Rohm & Haas Co.
3. The maximum linear rate occurs within the first minute of the reaction.
4. The millimolar extinction coefficient is cited in Keesey, J. (1987).
5. This assay is based on the cited references.

NOTES: (continued)

6. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.