

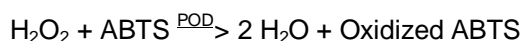
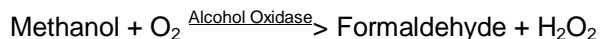


SIGMA QUALITY CONTROL TEST

Product Information

Enzymatic Assay of ALCOHOL OXIDASE (EC 1.1.3.13)

PRINCIPLE:



Abbreviations:

ABTS¹ = 2,2'-Azino-bis(3-Ethylbenzthiazoline-6-Sulfonic Acid)

POD = Peroxidase

CONDITIONS: T = 25°C, pH = 7.5, A_{405nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 100 mM Potassium Phosphate Buffer, pH 7.5 at 25°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 7.5 at 25°C with 1 M KOH.)
- B. 2 mM 2,2'-Azino-bis(3-Ethylbenzthiazoline-6-Sulfonic Acid) Solution (ABTS)
(Prepare 25 ml in Reagent A using 2,2'-Azino-bis(3-Ethylbenzthiazoline-6-Sulfonic Acid), Diammonium Salt, Sigma Prod. No. A-1888. **PREPARE FRESH.** Immediately before use, bubble O₂ gas through the solution for approximately 5 minutes.)
- C. 0.003% (w/w) Hydrogen Peroxide Solution
(Prepare 100 ml in deionized water using Hydrogen Peroxide, 30% (w/w) Solution, Sigma Prod. No. H-1009. **PREPARE FRESH.**)
- D. 1.0% (v/v) Methanol Solution
(Prepare 10 ml in deionized water using Methanol, Absolute, Sigma Stock No. 17-5. **PREPARE FRESH.**)

**Enzymatic Assay of ALCOHOL OXIDASE
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REAGENTS: (continued)

- E. Peroxidase² Enzyme Solution (POD)
(Immediately before use, prepare a solution containing 250 units/ml of Peroxidase, Sigma Prod. No. P-8250, in cold Reagent A.)

- F. Alcohol Oxidase Enzyme Solution
(Immediately before use, prepare a solution containing 0.1 unit/ml of Alcohol Oxidase in cold Reagent A.)

PROCEDURE:

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

| | Test | Blank |
|------------------|------|-------|
| Reagent B (ABTS) | 2.80 | 2.80 |
| Reagent E (POD) | 0.01 | 0.01 |

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

| | | |
|-------------------------------|-------|-------|
| Reagent A (Buffer) | ----- | 0.10 |
| Reagent F (Alcohol Oxidase) | 0.10 | ----- |
| Reagent C (Hydrogen Peroxide) | 0.01 | 0.01 |

Mix by inversion and monitor the $A_{405\text{nm}}$ until constant. Then add:

| | | |
|----------------------|------|------|
| Reagent D (Methanol) | 0.10 | 0.10 |
|----------------------|------|------|

Immediately mix by inversion and record the increase in $\Delta A_{405\text{nm}}$ for approximately 5 minutes. Obtain the $\Delta A_{405\text{nm}}/\text{minute}$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{405\text{nm}}/\text{min Test} - \Delta A_{405\text{nm}}/\text{min Blank})(3.02)(\text{df})}{(36.8)(0.1)}$$

3.02 = Total volume (in milliliters) of assay

df = Dilution factor

36.8 = Millimolar extinction coefficient of 2,2'-Azino-bis(3-Ethylbenzthiazoline-6-Sulfonic Acid) at 405 nm³

0.1 = Volume (in milliliters) of enzyme used

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CALCULATIONS:

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

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

UNIT DEFINITION:

One unit will oxidize 1.0 :mole of methanol to formaldehyde per minute at pH 7.5 at 25°C.

FINAL ASSAY CONCENTRATIONS:

In a 3.02 ml reaction mix, the final concentrations are 96 mM potassium phosphate, 2 mM 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid), 0.00001% (w/w) hydrogen peroxide, 0.033% (v/v) methanol, 2.5 units peroxidase and 0.01 unit alcohol oxidase.

REFERENCES:

Keeseey, J. (1987) *Biochemica Information*, 1st ed., p. 58, Boehringer Mannheim Biochemicals, Indianapolis, IN

Janssen, F.W. and Ruelius, H.W. (1968) *Biochimica et Biophysica Acta* **151**, 330-342

NOTES:

1. ABTS is a registered trademark of Boehringer Mannheim GmbH.
2. Peroxidase Unit Definition: One unit will form 1.0 mg purpurogallin from pyrogallol in 20 seconds at pH 6.0 at 20°C. In general, Peroxidase will use 2,2'-Azino-bis(3-Ethylbenzthiazoline-6-Sulfonic Acid) as an electron donor.
3. The millimolar extinction coefficient is cited in Keeseey, J. (1987).
4. This assay is based on the cited references.
5. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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