FT-607325

Alcohol Oxidase

Description

Catalog #:	*Name / Features*	*Storage:*
607325, 250EU 607326, 1 000EU 607327, 5 000EU 607328, 20 000EU 607329, 20 000EU Inquire for bulk quantities	**Alcohol Oxidase** Pichia Pastoris, Alcohol, Oxygen Oxidoreductase: E.C. 1.1.3.13 CAS Number: 9073-63-6 ; EC Number: 232-971-3 : MW: 630 000	-20°C (I)

Applications

Enzyme assays

Technical Information

Description: A wine-red solution of Alcohol Oxidase enzyme in buffered sucrose. The color changes reversibly to yellow in the presence of substrate under anoxic conditions. The color changes irreversibly to yellow if the enzyme is exposed to denaturing conditions.

Identity: *Pichia Pastoris*, Alcohol, Oxygen Oxidoreductase: E.C. 1.1.3.13

Reaction: $\text{Alcohol} + O_2 \xrightarrow{\text{Alcohol Oxidase}} \text{Aldehyde} + H_2O_2$

Activity: Alcohol Oxidase has a guaranteed minimum specific activity of 10 EU/mg protein and 1000 EU/ml of solution as determined using the assay described below. One unit of activity catalyzes one micromole of ethanol to aldehyde and hydrogen peroxide per minute in an air saturated solution at pH 7.5, at 25ºC.

Properties: **Specificity:** Alcohol Oxidase is specific for short-chain, linear aliphatic alcohols and oxidizes methanol and ethanol. The following are oxidized at decreasing velocity rate: methanol = ethanol, n-propanol, n-butanol. It also catalyzes the oxidation of allyl and propargyl alcohol, methyl and ethyl mercaptan and fromaldehyde at slower rates. Branched chain alcohols, C2-aldehydes or higher, ketones and organic acids are not substrates. However, some of these reagents can give false positive responses due to trace contamination with alcohol. Oxygen is the only known hydrogen acceptor.
At air saturation, the apparent KM for alcohol oxidase is 0.7 mM using methanol and 9 mM using ethanol as the substrate. In oxygen saturated solutions the K_M values are higher. The turnover number is 20 000 sec^{-1}.
The **optimum** temperature range is 40 – 45ºC. The pH optimum is 7.2 with a working range of 5.5 – 9.5.
Inactivation: freezing does not inactivate the enzyme. Compared to other oxidases, it is relatively resistant to p-chloromercuribenzoate, heavy metals and hydrogen peroxide. Hydrogen peroxide does not inactivate the enzyme at concentrations many times higher than those reported to inactivate previously-described yeast alcohol oxidases (Hansenula, Candida).
Solubility of the enzyme is inversely related to temperature; refrigeration will often reverse cloudiness in solutions where precipitation is occurring. The enzyme is freely soluble in greater than 0.1 M Phosphate buffer above pH 7.0. The enzyme reversibly precipitates at below 0.05 M Phosphate and below pH 7.0. These boundaries can be extended under certain pH, temperature and ionic conditions. Alcohol Oxidase has a molecular weight of approximately 630 000 and is composed of eight subunits, each having one molecule of bound azide. Inhibitors include azide, Cu^{++}, Ag^{+}, Hg^{++}, p-chloromercuribenzoate, hydroxylamine and NaF.

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Applications: Qualitative or quantitative determination of ethanol or methanol; alcohol removal; aldehyde or hydrogen peroxide production; and oxygen scavenging. Unlike present ADH based assays for ethanol in biological specimens, no labile cofactors or trapping agents are required and considerably less enzyme is used per assay. Quantitative determination of alcohol using an oxygen electrode (enzyme electrode) is also possible.
With or without catalase, alcohol oxidase can be used to remove alcohol or scavenge oxygen. Using dilute methanol, sensitive samples can be sterilized through release of the products formaldehyde and hydrogen peroxide. The enzyme can also be used to generate H2O2 in radio-iodine labeling procedures.

Formulation: Alcohol Oxidase enzyme in 60% Sucrose with 0.1 M Potassium Phosphate Buffer, pH 8.0.
The solution is a red color. The color serves as a visual indication that the enzyme is active. Should it be inactivated by exposure to denaturing conditions such as heat, extremes of pH, etc., the color irreversibly changes to yellow.

Storage: Store as a frozen solution at -20ºC. Stable at sub-zero temperatures for at least one year.

Packaging: Alcohol Oxidase is packaged in research quantities 250-5000units, and larger quantities (20 000 and 100 000 EU) on request. Shipped with dry ice.

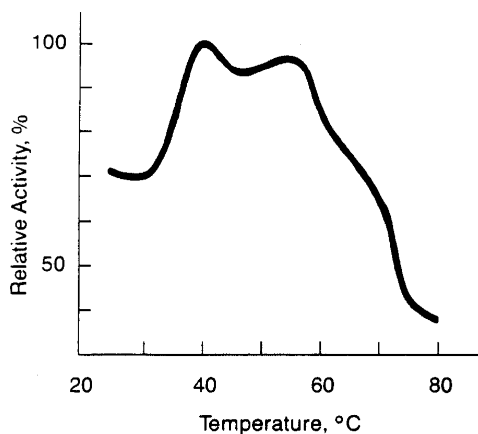

Figure 1. Temperature activity of 1:2000 dilution during 3 minutes incubation in 0.1 M phosphate buffer, pH 7.5.

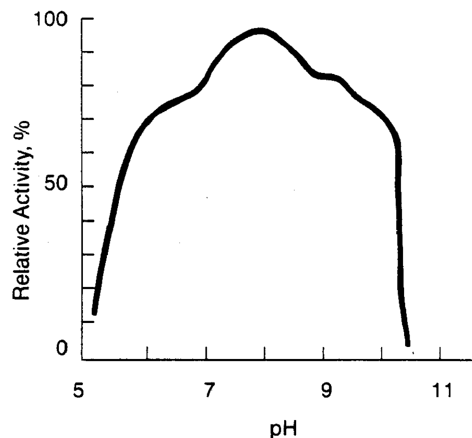

Figure 2. pH activity of 1:10,000 dilution in .025 molar citrate/ethylenediamine better determined using polaragraphic method at °C.

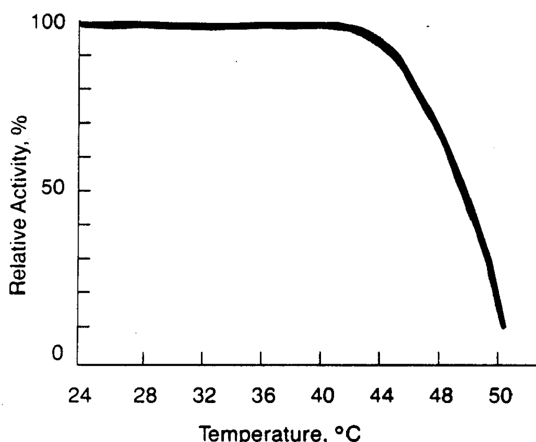

Figure 3. Thermal stability of 1:2000 dilution after 30 minutes incubation in 0.1 M phosphate buffer, pH 7.5.

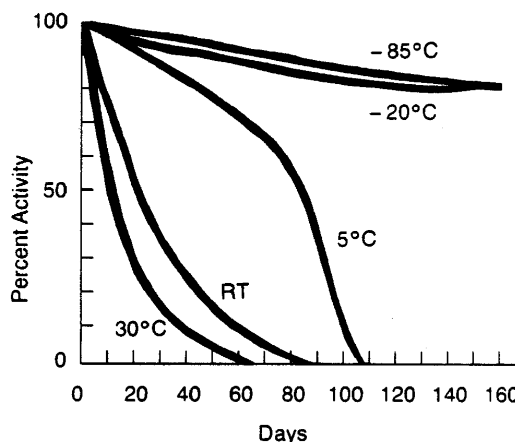

Figure 4. Long-term stability.

Relative activity (%) determined by ABTS method described in Technical Bulletin No. 1.

Directions for use

Method for Determining Alcohol Oxidase Activity by ABTS Assay

This method is provided in order to establish a standard method for the determination of Alcohol Oxidase enzyme activity in highly concentrated and viscous solutions. Because of the high viscosity of Alcohol Oxidase products, extra care must be taken in pipetting and mixing the dilutions used in the ABTS Activity Assay.

Materials:

1. Concentrated Alcohol Oxidase (500 to 1 500 EU/ml) in sucrose, warmed to room temperature.

2. ABTS Stock Solution
   - 16.0 mg ABTS (2,2'-Azino-bis(3-ethylbenz-thiazoline-6-sulphonic acid)) #UP423876
   - 2 ml Absolute Ethanol
   - 1 ml of 1 mg/ml Horseradish Peroxidase (150-2200 EU/mg) in distilled water #UP146500
   - 0.1 M Potassium Phosphate buffer, pH 7.5

   Completely dissolve the ABTS in a portion of the buffer (about 75 ml), then add the Ethanol and Peroxidase; make up to final volume of 100 ml.

3. 0.1 M Potassium Phosphate Buffer, pH 7.5

4. Positive displacement 100 µl pipette or hamilton syringe.

5. Recording spectrophotometer suitable for enzyme kinetics.
   Spectrophotometer temperature set to 30°C, wavelength set to 390 nm, light path of 10 mm.

Dilution Procedure:

The following procedure is for preparing dilutions of highly viscous materials for the ABTS assay. Non-viscous solutions may be assayed directly.

1. Duplicate dilutions should be tested.

2. Pipet 9.9 ml of 0.1 M Potassium Phosphate buffer into two test tubes.

3. To the first test tube, add 100 µl of Alcohol Oxidase solution using a 100 µl positive displacement pipette (1:100 dilution)
   a. Slowly draw sample into pipette, discharge sample, and immediately draw sample into pipette.
   b. Wipe off pipette, taking care not to remove any sample from the pipette due to tissue absorbency.
   c. Pipet sample into the first test tube, dipping the pipette tip into the buffer.
   d. Cover test tube with Parafilm, manually shake vigorously for 5 seconds, vortex for 5 seconds, and then shake vigorously again for 5 seconds.

4. To the second test tube, add 100 µl of the 1:100 dilution using a positive displacement pipette (1:10,000 dilution), repeating steps 3a through 3d.

5. Determine the Alcohol Oxidase activity of the 1:10 000 dilution using the standard ABTS Assay below.

ABTS Assay:

1. Pipet 2.5 ml of ABTS Stock Solution (at room temperature and saturated with air) into a 3 ml cuvette. Allow the cuvette to warm in the spectrophotometer for 3 minutes.

2. Pipet 25 µl of a 1:10 000 dilution of Alcohol Oxidase into the cuvette, cover and mix by inverting three times. Insert cuvette into spectrophotometer.

3. Record absorbance change for 3 minutes at 390 nm.

Calculations:

1. Calculate the slope (ΔE390nm/min) of the resultant curve from 30 to 180 seconds.

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2. Multiply the slope by 3.06 (extinction coefficient plus cuvette dilution factor) and by the initial dilution factor (10 000).

Notes:

1. Precisely weigh the ABTS. Slight variations in the amount can result in errors in the assay. ABTS solution should be made immediately before use.

2. The major errors associated with obtaining reproducible results with this Alcohol Oxidase Assay are in accurately pipetting and mixing the highly viscous Alcohol Oxidase products. Therefore, extreme care should be taken in pipetting and mixing the initial dilutions.

References:

1. Janssen, F.W., and H.W. Ruelius, Biochim. Biophys. Acta., v. 151, 303 (1968).
2. Tani, Y., T. Miya, H. Nishikawa, and K. Ogata, Agr. Biol. Chem., v. 36, 68 (1972).
3. Fujii, T., and K. Tonomura, Agr. Biol. Chem., v. 36, 2297 (1972).
4. Tani, Y., T. Miya, and K. Ogata, Agr. Biol. Chem., v. 36, 76 (1972).
5. Sahm, H., and F. Wagner, Eur. J. Biochem., v. 36, 250 (1973).
6. Kato, N., Y. Tani, and K. Ogata, Agr. Biol. Chem., v. 38, 675 (1974).
7. Kato, N., Y. Omori, Y. Tani, and K. Ogata, Eur. J. Biochem, v. 64, 341 (1976).

Other Information

Related products:

#60506 Alcohol Oxidase from Candida Boidinii

Please contact InterBioTech – Interchim for any other information

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