HEXOSE OXIDASE from *CHONDRUS CRISPUS* expressed in *HANSENULA POLYMORPHA*

New specifications prepared at the 63rd JECFA (2004) and published in FNP 52 Add 12 (2004). An ADI "not specified" was established at the 63rd JECFA (2004).

SYNONYMS HOX

SOURCES Produced by a submerged fermentation of a pure culture of a non-pathogenic and nontoxigenic genetically modified strain of *Hansenula polymorpha* containing the hexose oxidase gene derived from *Chondrus crispus*. The enzyme is produced intracellularly and upon cell disruption is released into the fermentation broth and is subsequently separated from the yeast cells and subjected to ultrafiltration and diafiltration to obtain the concentrated hexose oxidase. It is then spray-dried onto a suitable food-grade carrier such as wheat starch.

Active principles: Hexose oxidase

Systematic names and numbers: D-hexose:oxygen 1-oxidoreductase; EC 1.1.3.5; CAS No. 9028-75-5

Reactions catalyzed: Oxidation of hexose sugars to their corresponding lactones and hydrogen peroxide

DESCRIPTION Off-white to brownish micro-granules

FUNCTIONAL USES Enzyme preparation.
Used in bread dough to strengthen the gluten network; in products like shredded cheese, potato chips, egg white powder and whey protein isolates to minimize Maillard reactions; in cottage cheese and tofu to facilitate curd formation and in products like ketchup, mayonnaise and salad dressings to scavenge oxygen.

GENERAL SPECIFICATIONS Must conform to the *General Specifications for Enzyme Preparations used in Food Processing* (see Volume Introduction)

CHARACTERISTICS

IDENTIFICATION

Hexose oxidase activity: The sample shows hexose oxidase activity
See description under TESTS

TESTS

Hexose oxidase activity

Principle
Hexose oxidase catalyses the formation of hydrogen peroxide and glucono-delta-lactone from glucose and oxygen. Hydrogen peroxide subsequently reacts with 2, 2'-azino-bis(3-ethylbenzthiazolin-6-sulfonic acid) (ABTS), resulting in the development of a green colour. This reaction is catalysed by the enzyme peroxidase. The colour intensity is measured spectrophotometrically at 405 nm.

The activity unit HOX is defined as the amount of enzyme that catalyses the formation of 1 μmole of H_2O_2 per minute at 25 °.

Reagents

0.1M Phosphate buffer, pH 6.3: Dissolve 22.82 g $K_2HPO_4 \cdot 3H_2O$ in approx. 800 ml water. The pH is adjusted to 6.3 with conc. HCl and to 1000 ml with water.

Reagent 1 (Glucose (55 mM) in 0.1 M phosphate buffer, pH 6.3): Dissolve 5.44 g glucose monohydrate ($C_2H_{12}O_6 \cdot H_2O$ D(+)) in 400 ml freshly made 0.1 M phosphate buffer. Adjust to pH 6.3 with conc. HCl. And to 500 ml with 0.1 M phosphate buffer.

Reagent 2 (ABTS stock solution): Weigh 500 mg ABTS into a 100 ml measuring flask and dilute to volume with water. Store in 1.5 ml volumes.

Reagent 3(Peroxidase (0.1 mg/ml) in 0.1 M phosphate buffer, pH 6.3): Dissolve 10 mg peroxidase in 100 ml phosphate buffer. Store in 1.5 ml volumes.

Procedure

Substrate preparation: Pipette 1.0 ml Reagent 2 and 1.0 ml Reagent 3 into a 25 ml measuring flask and make up to volume with Reagent 1. The substrate is held for 30 min in a water bath at 25 °.

Standard curve for H_2O_2 assay: Dilute 0.2 ml H_2O_2 (concentration determined) with water to 1000 ml. This gives the control sample a peroxide concentration of approx. 2 µmol/ml.

The following standard curve is prepared:

Approx. peroxide concentration (µmol/ml)	*Dilution B (ml)*	*Water (ml)*
0.000	0.0	20.0
0.050	0.5	19.5
0.100	1.0	19.0
0.200	2.0	18.0
0.400	4.0	16.0
0.600	6.0	14.0
0.800	8.0	12.0

Measuring the standard curve: Pipette 50 µl from each concentration (0-0.8 µmol/ml) into a disposable microcuvette and add 950 µl of substrate. Measure the absorbance after 5 min at 405 nm. Zero the spectrophotometer with the sample containing 0 µmol/ml peroxide.

Sample preparation

Make duplicate measurements of the activity. Weigh out the enzyme as follows:

Sample weight (g) = 31/expected activity per gram

Dissolve the samples in 100 ml volumetric flasks with phosphate buffer and mix on a magnetic stirrer for 20 min. Fill the flasks with buffer, and filter turbid samples through syringe filters (Cameo 25 A, 0.45µ or equivalent). Subsequent dilutions are made in phosphate buffer.

Measurement

Measure the samples within 30 min of the final dilution. Zero the spectrophotometer with a sample of buffer. Add 50 µl of diluted sample to a disposable microcuvette. Place the cuvette in the photometer, and when 950

μl substrate is added to the cuvette, start the measurement of optical density.

Measure the optical density at 405 nm at 5 second intervals for 1 minute. Plot the optical density as a function of time. Determine the slope (OD/min) of the curve for the interval 0.25 to 0.75 min. The maximal OD at which a linear response is obtained is approx. 2.0 because the amount of ABTS becomes a limiting factor at higher OD.

Calculations

The activity of powder samples is determined as follows:

$$HOX / g = \frac{D \cdot act \cdot 100}{Std.slope \cdot weight}$$

Where:

D =	Dilution
act =	slope in OD/min of the sample
weight =	grams of spray dried powder suspended in 100 ml K_2HPO_4 buffer
Std. slope =	slope in OD/μmol/ml of the standard curve