

# Ascorbate Oxidase

Catalogue No. 70-6141-20

E.C. Number 1.10.3.3

## ORIGIN

*Cucurbita pepo medullosa*

## SPECIFICATIONS

Appearance . . . . . Light tanish, brownish, greyish to blue green free flowing powder

Activity . . . . . 156.0 to 624.0 U/mg powder at 37°C

Protein concentration. . . . .  $\geq 40.0\%$

Contaminants:

o Catalase . . . . .  $<0.096\%$  by activity

o Adenylate Kinase . . . . .  $\leq 0.5$  U/mg powder

o Glucose Oxidase . . . . .  $<0.002$  U/mg powder

o Cholesterol Oxidase . . . . .  $<0.002$  U/mg powder

o Uricase . . . . .  $<0.002$  U/mg powder

o Lactate Oxidase . . . . .  $<0.002$  U/mg powder

Stability. . . . . Stable for 1 year stored at  $-20^{\circ}\text{C}$

## ASSAY PRINCIPLE

Ascorbic acid oxidase (AAO) catalyses the following reaction:



## APPLICATION

AAO can be used in clinical tests for determining levels of ascorbic acid in blood or for the removal of interference effects caused by ascorbic acid in clinical analysis.

## UNIT DEFINITION

One unit of activity is defined as the amount of enzyme that will catalyse the oxidation of 1.0 micromole of ascorbic acid per minute at  $37^{\circ}\text{C}$  under the standard assay method conditions (available on request).

**TABLE 1** Temperature factors for unit conversion

Assay Temp	Factor Realitve to 37°C Result
25°C	0.58
30°C	0.72
37°C	1.00
45°C	1.18

Note: Temperature can influence the level of available oxygen in the reaction mixture.

**CHARACTERISTICS**

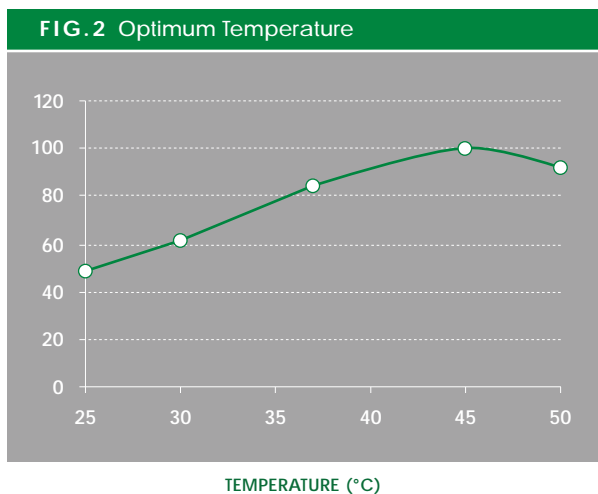
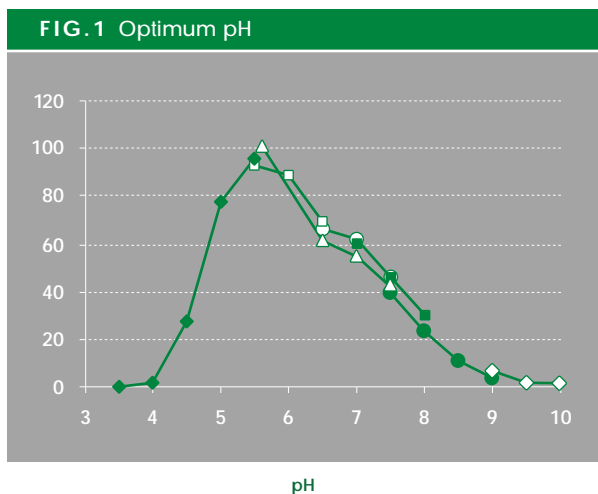
AAO is formulated in buffer without any additional additives. Its main characteristics are as follows:

- Native structure<sup>(1)</sup> ..... 70kD subunit arranged as a tetramer, glycosylated, 4 Cu atoms per subunit
- K<sub>m</sub> value (calculated using Eadie-Hofstee) ..... 2 x 10<sup>-4</sup> M (L-Ascorbic Acid)
- Optimum pH (Fig. 1) ..... pH 5.5 to 6.0
- Optimum temperature (Fig. 2) ..... 45°C
- Stable pH range (Fig. 3) ..... pH 5.5 to 10.0 (25°C for 20 hours)
- Thermal stability (Fig. 4) ..... 60 min at 50°C and below
- Lyophilised stability ..... Up to 50°C (pH 7.0 for 15 mins)

**Substrate Specificity**

Substrate specificity was tested in-house by replacing ascorbic acid with alternative substrates in the AAO assay i.e. at 0.5 mM concentration.

TABLE 2 Substrate specificity	
Substrate	% of Ascorbic Acid Activity
L-Ascorbic acid	100
D-Araboascorbic acid	47
Hydroquinone	<0.1
Pyrogallol	<0.1
Catechol	<0.1



- ◆ SODIUM ACETATE    □ MES    ○ MOPS    ■ TRIS
- △ POTASSIUM PHOSPHATE    ● BICINE    ◇ CHES

