ASCORBATE OXIDASE [ASOM]

from *Acremonium* sp.
(L–Ascorbate: oxygen oxidoreductase, EC 1.10.3.3)

L– Ascorbic Acid + 1/2 O_2 ⟶ Dehydroascorbic Acid + H_2O

Preparation and Specification

Appearance : Light blue amorphous powder, lyophilized
Specific activity : More than 200 U/mg solid

Properties

Substrate specificity	: See Table 1	
Molecular weight	: 80 kDa (gel filtration)	
Isoelectric point	: pH 4.0	
Michaelis constants	: Ascorbic acid (pH 7.0) 1.0×10^{-4} M	
	Ascorbic acid (pH 4.0) 3.8×10^{-4} M	
Optimum pH	: 4.0–4.5 (Acetate buffer)	Figure 1
pH stability	: 6.0–10.0 (30℃, 24 hr)	Figure 2
Thermal stability	: Stable at 50℃ and below (pH 7.0, 10 min)	Figure 3
Liquid stability	: See Figure 4	
Effect of metal ions	: See Table 2	
Stabilizers	: BSA, Mannitol	

Applications for Diagnostic Test

This enzyme is useful for **avoidance from interference of ascorbic acid** on diagnostic assay such as blood, uric acid, TG, TC and creatinine.

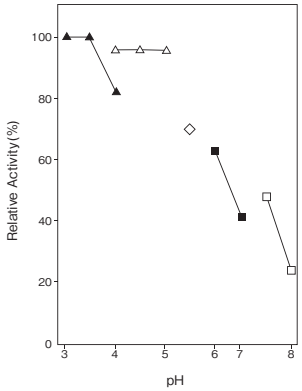
Table 1. Substrate specificity

Substrate	Relative activity (%)
L-Ascorbate	100
l-Naphthol	0
Hydroquinone	0
Catechol	0
Pyrogallol	0

Table 2. Effect of metal ions on ASOM activity

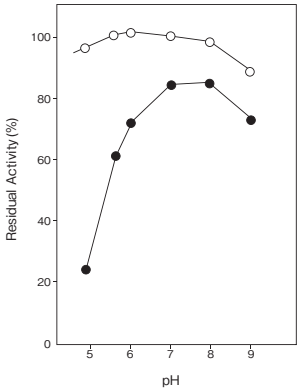
Metal ion	Concentration	Relative activity (%)
None		100
KCl	10 mM	100
NaCl	10 mM	100
EDTA	1 mM	100

Fig.1 pH Optimum



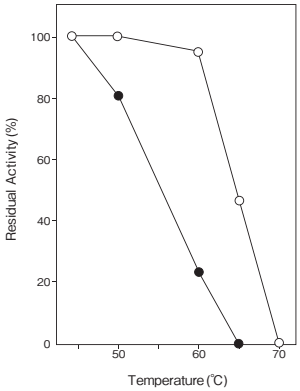
□ : Tris-HCl
■ : Phosphate
◇ : MES
△ : Acetate
▲ : Glycine-HCl

Fig.2 pH Stability



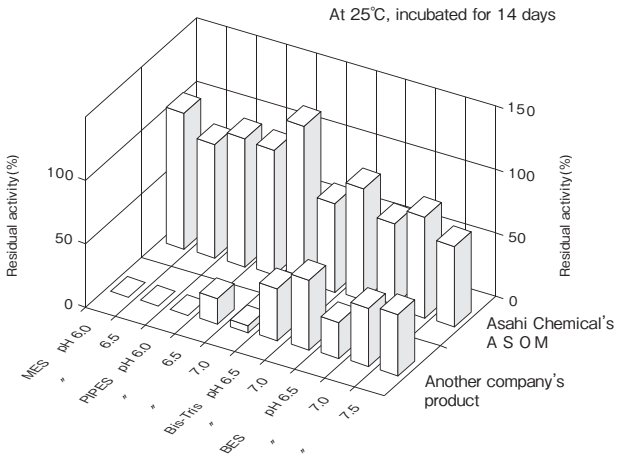
32℃, 24 hrs.
○ : ASOM
● : ASCORBATE OXIDASE from Cucumber

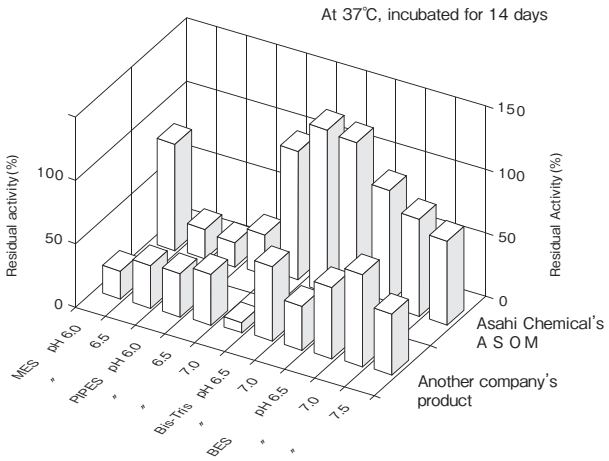
Fig.3 Thermal Stability



50 mM phosphate buffer (pH 7.0) containing 0.1% BSA, 1 mM EDTA 10 min.
○ : ASOM
● : ASCORBATE OXIDASE from Cucumber

Fig.4 Liquid stability of ASOM (Buffer, pH)





Assay

■ Principle

The assay is based on the decrease in absorbance at 245 nm as ascorbic acid is oxidized in the following reaction:

$$\text{Ascorbic acid} + 1/2\ O_2 \xrightarrow{\text{ASOM}} \text{Dehydroascorbic acid} + H_2O$$

■ Unit definition

One unit is defined as the amount of enzyme which oxidizes 1 μmole of ascorbate to dehydroascorbate per minute at 30 ℃ under the conditions specified in the assay procedure.

■ Reagents

1. Reaction mixture

Dilute substrate solution for stock [1] with dilution buffer [2] to make a 20-fold solution.

1): Substrate solution for stock (10 mM L-ascorbic acid solution)
Dissolve 176 mg of L-ascorbic acid and 37 mg of EDTA with 100 ml of 1mM HCl.
EDTA: Ethylenediamine tetraacetic acid

2): Dilution buffer
90mM KH_2PO_4-5mM Na_2HPO_4 buffer containing 0.45 mM EDTA

2. Reaction stopper
   0.2 N HCl solution
3. Enzyme dilution buffer
   10 mM Na_2HPO_4 solution containing 0.05% (W/V) BSA
4. Reagents
   L–Ascorbic acid: Wako Pure Chemical Industries, Ltd.
   Special grade # 012–04802
   EDTA (2Na·2H_2O): KISHIDA CHEMICAL Co., Ltd.
   #060–29133
   BSA: Millipore Fraction V pH 5.2 #81–053

■ **Enzyme solution**

Accurately weigh about 20 mg of the sample and add enzyme dilution buffer to make a total of 20 ml. Dilute it with enzyme dilution buffer to adjust the concentration to within 0.08–0.35 U/ml.

■ **Procedure**

1. Pipette accurately 1.0 ml of reaction mixture into a small test tube and preincubate at 30℃.
2. After 5 min, add exactly 100 μl of enzyme solution and mix to start the reaction at 30℃.
3. At 10 min after starting the reaction, add 3.0 ml of the reaction stopper to stop the reaction.
   ※ In the case of a test blank, add 100 μl of enzyme dilution buffer after adding reaction stopper in place of enzyme solution.
4. Measure the absorbance at 245 nm.
   Absorbance sample : As
   blank : Ab
   $0.100Abs \leqq \Delta A = Ab - As \leqq 0.420Abs$

■ **Calculation**

$$\text{Activity (U/mg of powder)} = \frac{\triangle A/5}{10.0} \times \frac{4.10}{0.10} \times \frac{1}{X}$$

10.0 : millimolar extinction coefficient of ascorbic acid at 245 nm at pH 1.0 ($cm^2/\mu mole$)
5 : reaction time (min)
4.10 : final volume (ml)
0.10 : volume of enzyme solution (ml)
X : concentration of the sample in enzyme solution (mg/ml)

Storage

Storage at −20℃ in the presence of a desiccant is recommended.

References

1. Murao, S., et al. (1991) Agric. Biol. Chem., **55 (6)** , 1693–1694.
2. Nakamura, T., Makino, N. and Ogura, Y. (1968) J. Biochem., **64**, 189.
3. Aikazyan, V. Ts. and Nalbandyan, R. M. (1979) FEBS Lett, **104**, 127.
4. White, G. A. and Smith, F. G. (1961) Nature, **190**, 187.

ASOM 活性測定法 (Japanese)

Ⅰ. 試薬液

1. 保存基質溶液 (10mM L–アスコルビン酸)
   L–アスコルビン酸 176mg と EDTA 37mg を 1mM HCl 100ml で溶解する。
2. 反応試薬混合液
   上記の保存基質溶液を希釈用液※) で 20 倍に希釈する。
   ※): 希釈用液
   0.45mM EDTA を含む 90mM KH_2PO_4–5mM Na_2HPO_4 溶液
3. 反応停止液
   0.2N HCl 液
4. 酵素溶解希釈用液
   0.05% (W/V) BSA を含む 10mM Na_2HPO_4 溶液
5. 試薬
   L–アスコルビン酸:
   和光純薬工業製 特級 #012–04802
   EDTA (エチレンジアミン四酢酸·2Na·2H_2O):
   キシダ化学社製 #060–29133
   BSA: Millipore 社製 Fraction V pH5.2 #81–053

Ⅱ. 酵素試料液

検品約 20mg を精密に量り、酵素溶解希釈用液に溶解して全容 20ml とする。
その液を酵素溶解希釈用液で 0.08～0.35U/ml 濃度となるように適宜希釈する。

Ⅲ. 測定操作法

1. 小試験管に反応試薬混合液 1.0ml を正確に分注して 30℃で予備加温する。
2. 5 分経過後、酵素試料液 100 μl を加えて混和し、30℃で反応を開始する。
3. 5 分経過後、反応停止液 3.0ml を加えて混和し、反応を停止する。
   ※盲検は反応停止後に酵素試料液 100 μl を加える。
4. 245nm における吸光度を測定する。
   求められた吸光度を試料液は As、盲検液は Ab とする。
   $0.100Abs \leqq \Delta A = Ab - As \leqq 0.420Abs$

Ⅳ. 計算

$$\text{活性 (U/mg)} = \frac{\Delta A/5}{10.0} \times \frac{4.10}{0.10} \times \frac{1}{X}$$

10.0 : pH 1 の条件でアスコルビン酸の 245nm におけるミリモル分子吸光係数 ($cm^2/\mu mole$)
5 : 反応時間 (min)
4.10 : 反応総液量 (ml)
0.10 : 反応に供した酵素試料液量 (ml)
X : 酵素試料液中の検品濃度 (mg/ml)