

ASCORBATE OXIDASE [ASOM]

from *Acremonium* sp.

(L-Ascorbate: oxygen oxidoreductase, EC 1.10.3.3)



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	: 6.0–9.0 (30°C, 24 hr)	Figure 2
Thermal stability	: Stable at 50°C 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

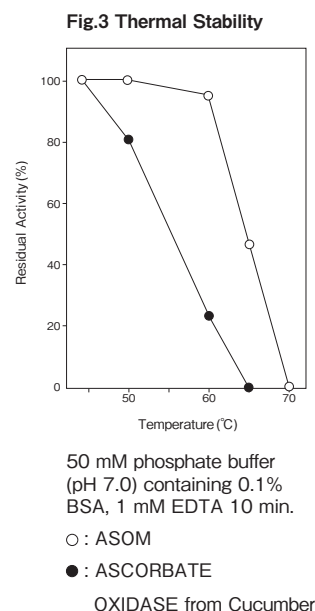
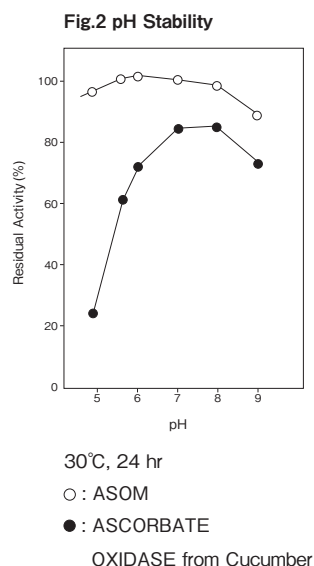
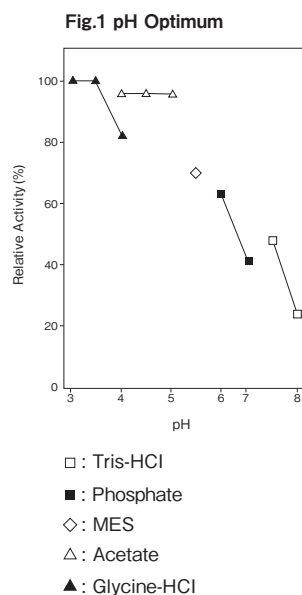
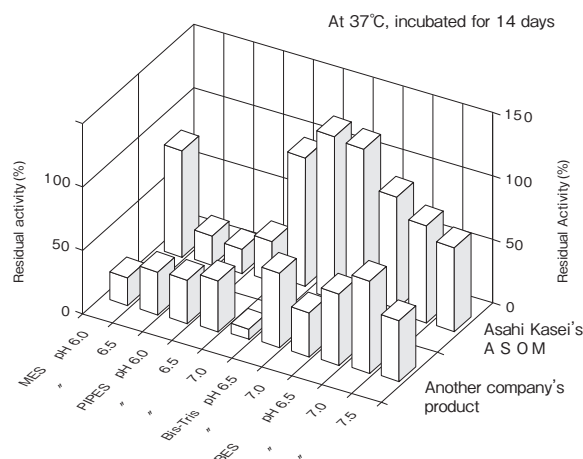
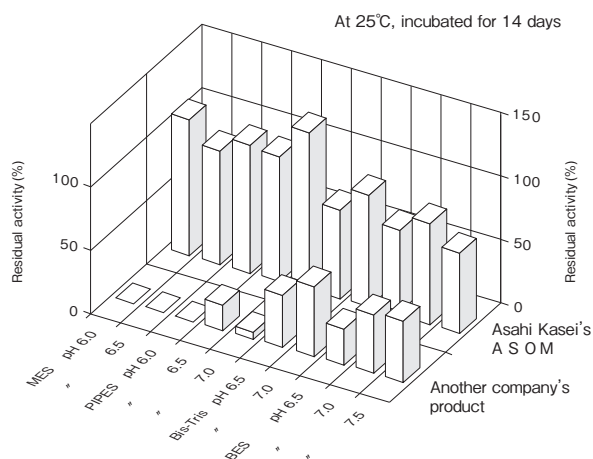


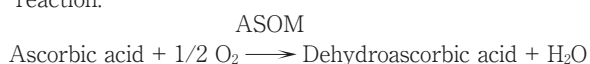
Fig.4 Liquid stability of ASOM (Buffer, pH)



Assay

Principle

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



Unit definition

One unit is defined as the amount of enzyme which oxidizes 1 μ mole of ascorbate to dehydroascorbate per minute at 30 °C 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 KH₂PO₄-5mM Na₂HPO₄ buffer containing 0.45 mM EDTA

2. Reaction stopper
0.2 N HCl solution
3. Enzyme dilution buffer
10 mM Na₂HPO₄ solution containing 0.05% (W/V) BSA
4. Reagents
L-Ascorbic acid:
FUJIFILM Wako Pure Chemical Corporation
Special grade # 012-04802
EDTA (2Na·2H₂O): 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°C.
2. After 5 min, add exactly 100 μl of enzyme solution and mix to start the reaction at 30°C.
3. At 5 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 : A_s
blank : A_b
0.100Abs ≤ ΔA = A_b - A_s ≤ 0.420Abs

■ Calculation

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

- 10.0 : millimolar extinction coefficient of ascorbic acid at 245 nm at pH 1.0 (cm²/μmole)
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°C 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 KH₂PO₄-5mM Na₂HPO₄ 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 Na₂HPO₄ 溶液
5. 試薬
L-アスコルビン酸:
富士フィルム和光純薬製 特級 #012-04802
EDTA (エチレンジアミン四酢酸·2Na·2H₂O):
キシダ化学製 #060-29133
BSA: Millipore 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°C 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°C 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 A_s、盲検液は A_b とする。

$$0.100\text{Abs} \leq \Delta A = A_b - A_s \leq 0.420\text{Abs}$$

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 (cm²/μmole)
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)