

ASCORBATE OXIDASE [ASOK]

from Microorganism
(L-Ascorbate: oxygen oxidoreductase, EC 1.10.3.3)



Preparation and Specification

Appearance : Light blue to blue lyophilizate
Specific activity : More than 200 U/mg solid

Properties

Molecular weight	: 88 kDa (SDS-PAGE)	
Isoelectric point	: 5.1 (estimated from amino acid sequence)	
Michaelis constant	: 4.3×10^{-4} M	
Optimum pH	: 5.8-6.3	Figure 1
pH stability	: 3.2-10.0 (30°C, 24 hr)	Figure 2
Thermal stability	: Stable at 60°C and below (pH 7.0, 10 min)	Figure 3
Storage stability	: Stable at least one year at -20°C	Figure 4
Solid stability	: See Figure 5	
Effect of metal ions	: See Table 1	
Effect of detergents	: See Table 2	
Light stability	: See Table 3	
Stabilizers	: BSA, Mannitol, Trehalose	

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 Effect of metal ions on ASOK activity

Metal ion (1 mM)	Relative activity (%)
None	100
MgCl ₂	97
MgSO ₄	98
ZnCl ₂	95
ZnSO ₄	100
NaCl	97
NH ₄ Cl	96
BaCl ₂	96
NiCl ₂	95
CoCl ₂	96
MnCl ₂	99
LiCl ₂	96
KCl	97
CaCl ₂	99
FeCl ₂	97

Table 2 Effect of detergents on ASOK stability

Detergent (0.1%)	Residual activity (%)		
	0°C	65°C	70°C
None (0.1% BSA)	100	71	28
TritonX-100	100	81	41
Emulgen 1108	100	81	40
Emulgen 1118S-70	100	85	42
Emulgen 1150S-60	100	81	41
AdekatoI TN-100	100	84	42
Newcol 710	100	83	45
Newcol 710-F	100	83	46
Newcol 714-F	100	85	44
Newcol 707	100	87	47

After 10 min of incubation at each temperature.

Table 3 Light stability

Light source	Residual activity (%)	
	0 h	24 h
LED	100	102
Fluorescent	100	101
UV	100	74

After 24 hours of incubation.

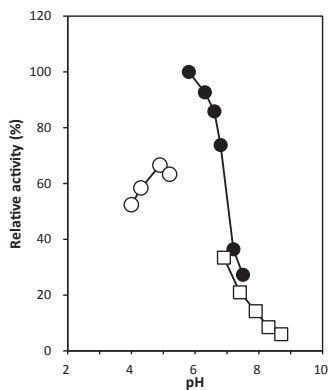


Fig. 1 Optimum pH

- : Acetate buffer
- : Phosphate buffer
- : Tris-HCl buffer

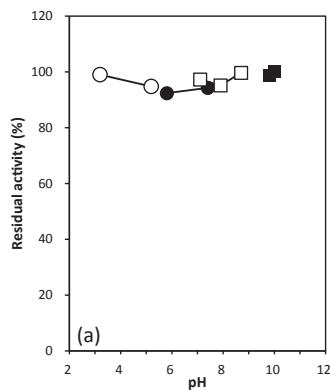
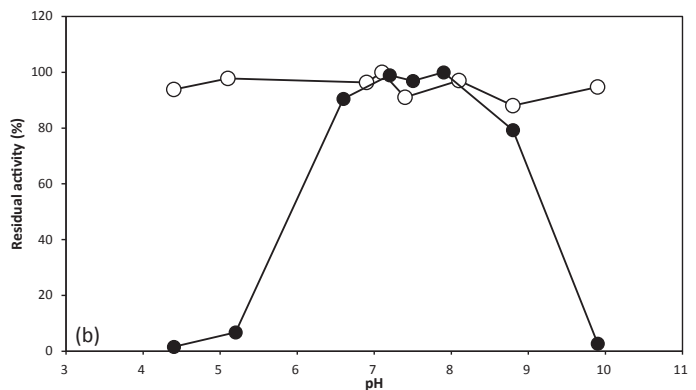


Fig. 2 pH stability

- (a) 30°C, 24 hours
- : Acetate buffer
- : Phosphate buffer
- : Tris-HCl buffer
- : Glycine-NaOH buffer



(b) 37°C, 24 hours

- : ASOK
- : ASO from *Cucumis* sp.

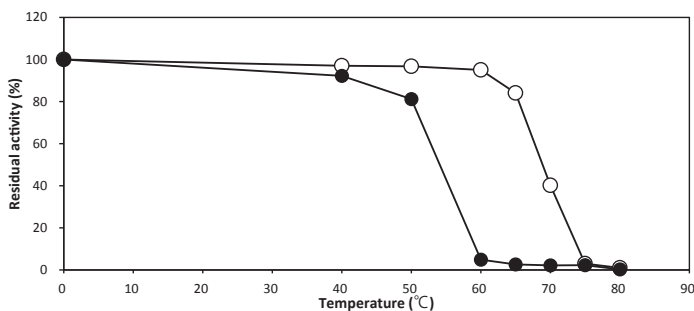


Fig. 3 Thermal stability

- pH 7.0, 10 min
- : ASOK
- : ASO from *Cucumis* sp.

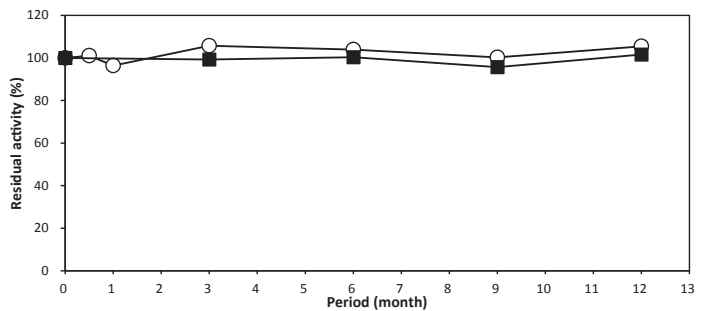


Fig. 4 Storage stability

- : -20°C
- : 5°C

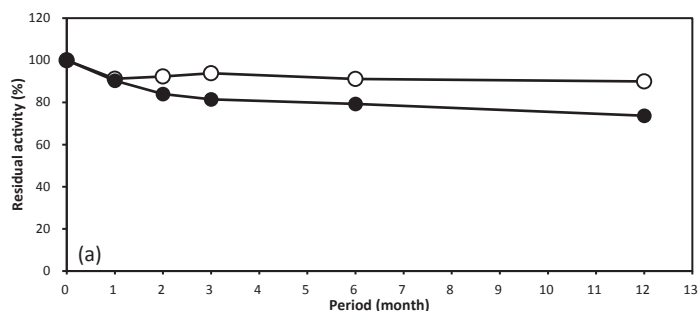
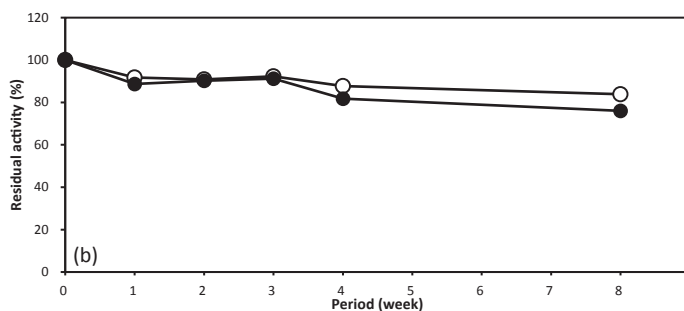


Fig. 5 Solid stability

- (a) 25°C
 ○ : ASOK
 ● : ASO from *Cucumis* sp.



- (b) 37°C
 ○ : ASOK
 ● : ASO from *Cucumis* sp.

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 stock¹⁾ with dilution buffer²⁾ 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

- Pipette accurately 1.0 ml of reaction mixture into a small test tube and preincubate at 30°C.
- After 5 min, add exactly 100 μ l of enzyme solution and mix to start the reaction at 30°C.
- 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 μ l of enzyme dilution buffer after adding reaction stopper in place of enzyme solution.
- Measure the absorbance at 245 nm.

Absorbance	sample : As
	blank : Ab
$0.100\text{Abs} \leq \Delta A = A_b - A_s \leq 0.420\text{Abs}$	

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/ μ 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°C in the presence of a desiccant is recommended.

References

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

ASOK 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:
富士フイルム和光純薬製 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 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 A_s、盲検液は A_b とする。

$$0.100A_{bs} \leq \Delta A = A_b - A_s \leq 0.420A_{bs}$$

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)