(Diagnostic Reagent Grade) ASAHI KASEI ENZYMES T−248

ASCORBATE OXIDASE [ASOK]

from Microorganism
(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 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℃, 24 hr)	Figure 2
Thermal stability	: Stable at 60℃ and below (pH 7.0, 10 min)	Figure 3
Storage stability	: Stable at least one year at -20℃	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.

A

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_4Cl	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℃	65℃	70℃
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
Adekatol 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.

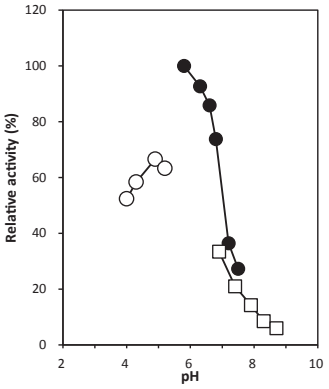


Fig. 1 Optimum pH

○ : Acetate buffer
● : Phosphate buffer
□ : Tris-HCl buffer

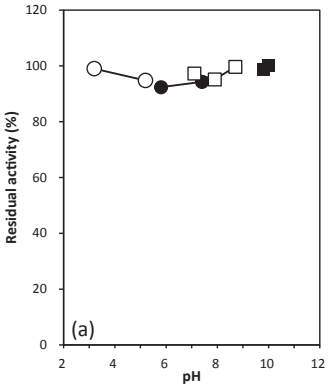
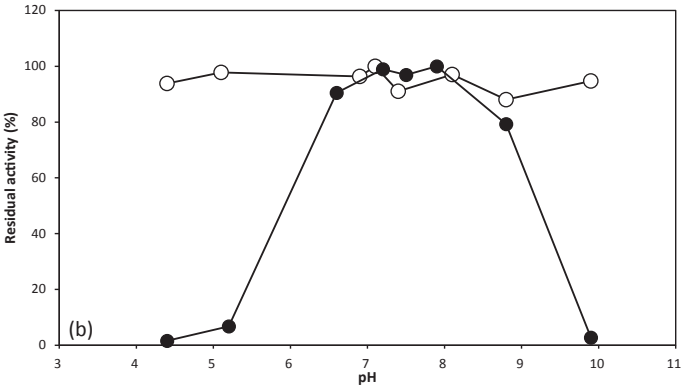


Fig. 2 pH stability

(a) 30℃, 24 hours
○ : Acetate buffer
● : Phosphate buffer
□ : Tris-HCl buffer
■ : Glycine-NaOH buffer



(b) 37℃, 24 hours
○ : ASOK
● : ASO from *Cucumis* sp.

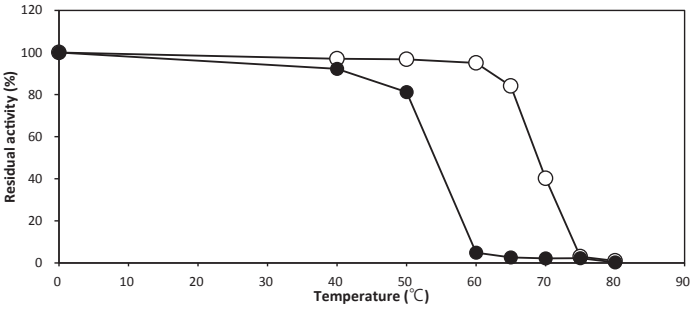


Fig. 3 Thermal stability

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

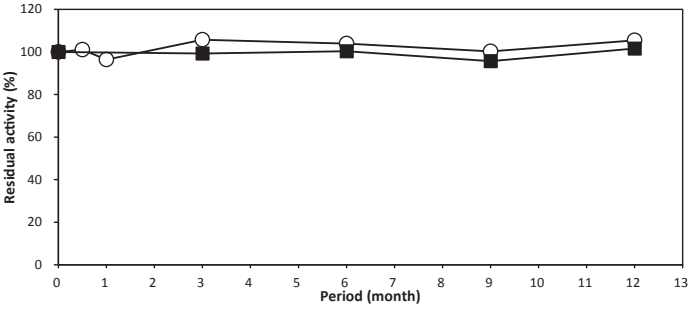
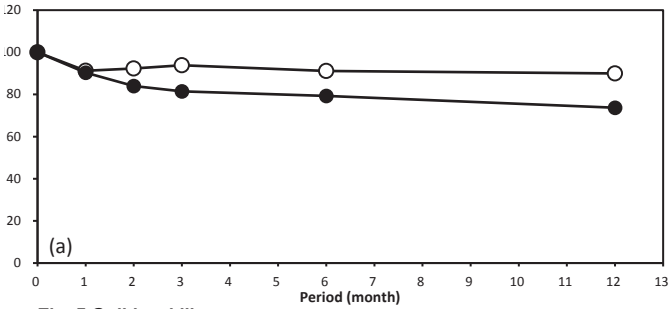


Fig. 4 Storage stability

○ : -20℃
■ : 5℃



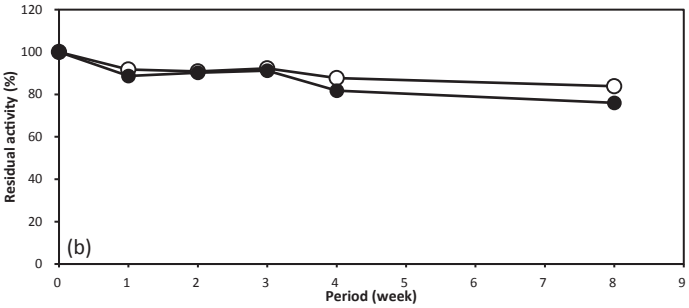


Fig. 5 Solid stability

(a) 25℃
○ : ASOK
● : ASO from *Cucumis* sp.

(b) 37℃
○ : 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:

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

■ 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:
   FUJIFILM Wako Pure Chemical Corporation
   Special grade # 012-04802
   EDTA ($2Na \cdot 2H_2O$): 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 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 : As
   blank : Ab
   $0.100\text{Abs} \leqq \Delta A = Ab - As \leqq 0.420\text{Abs}$

■ 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.

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 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:
   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･$2H_2O$）:
   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 As、盲検液は Ab とする。

$$0.100\text{Abs} \leqq \Delta A = Ab - As \leqq 0.420\text{Abs}$$

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　（$cm^2/\ \mu mole$）
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）