



重组抗坏血酸氧化酶 (ASO)

产品介绍

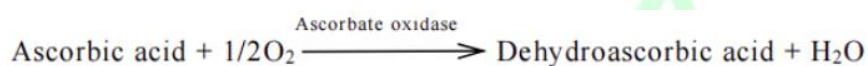
Ascorbate oxidase also known as vitamin C oxidase, is a REDOX enzyme involved in the regulation of extracellular matrix. Ascorbate oxidase catalyzes the reaction of ascorbic acid and oxygen to produce dehydroascorbic acid.

Application

It catalyzes the oxidation of ascorbic acid to produce water and dehydroascorbic acid, and is used for in vitro research.

Assay procedure

Principle



The disappearance of ascorbic acid is measured at 245nm by spectrophotometry.

Unit definition

One unit causes the decrease of one micromole of ascorbic acid per minute under the conditions described below.

Reagents

A. Ascorbic acid solution: 1.0mM [Dilute the stock solution (10mM) to 10-fold volume with 0.2M KH₂PO₄ solution containing 1.0mM EDTA.] (Prepare freshly)

Stock solution: 176mg L-ascorbic acid (MW=176.13) / 100 ml of 1.0mM HCl solution containing 1.0mM EDTA (Stable for one month if store at 0~5°C)

B. Na₂HPO₄ solution: 10mM

C. HCl solution: 0.2N

D. Enzyme diluent: 10mM Na₂HPO₄ solution containing 0.05% BSA. (Should be freshly prepared)

Procedure

1. Prepare the following reaction in a test tube and equilibrate at 30°C for about 5 minutes.

Substrate solution	0.5ml	(A)
Na ₂ HPO ₄ solution	0.5ml	(B)



(pH of the reaction mixture should be 5.6.)

Concentration in assay mixture

KH ₂ PO ₄	82mM
Na ₂ HPO ₄	5.5mM
Ascorbic acid	0.45mM
EDTA	0.45mM
BSA	45.4μg/ml

2. Add 0.1ml of the enzyme solution* and mix.

3. After exactly 5 minutes at 30°C, add 3.0ml of HCl solution (C) to stop the reaction and measure the optical density at 245nm against water (OD test). At the same time, prepare the blank by first mixing the reaction mixture with 3.0ml of HCl solution (C) after 5 min-incubation at 30°C, followed by adding the enzyme solution (OD blank).

*Dissolve the enzyme preparation in ice-cold enzyme diluent water (more than 60U/ml) and dilute to 0.15~0.25U/ml with the ice-cold enzyme diluent (D), immediately before assay.

Calculation

Activity can be calculated by using the following formula:

$$\text{Volume activity (U/ml)} = \frac{\Delta \text{OD}/\text{min}(\text{OD}_{\text{blank}} - \text{OD}_{\text{test}}) \times V_t \times \text{df}}{10.0 \times 1.0 \times t \times V_s} = \Delta \text{OD} \times 0.820 \times \text{df}$$

V_t: Total volume (4.1ml)

V_s: Sample volume (0.1ml)

10.0: Millimolar extinction coefficient of ascorbic acid under the assay conditions at pH1.0 (cm²/μmol)

1.0: Light path length (cm)

t: Reaction time (5 minutes)

df: Dilution factor

C: Enzyme concentration in dissolution (C mg/ml)