Dehydroascorbate Reductase Assay Kit

Catalog No.: abx298820

Size: 100 Assays

Storage: Store all components at 4°C in the dark.

Application: For quantitative detection of Dehydroascorbate Reductase activity in tissue homogenates and cell lysates.

Introduction: Dehydroascorbate Reductase (DHAR) is an enzyme found in plants and micro-organisms. It catalyzes the reduction of dehydroascorbate (DHA) to ascorbate (AsA) in the presence of glutathione, which is oxidized to glutathione disulfide.

Abbexa's Dehydroascorbate Reductase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Dehydroascorbate Reductase activity. The concentration of AsA can be calculated by measuring the absorbance at 265 nm, from which the enzyme activity can be calculated.

Kit components

1. 96 well microplate
2. Assay Buffer: 4 × 30 ml
3. Reaction Buffer: 20 ml
4. Substrate 1: 1 vial
5. Substrate 2: 1 vial

Materials Required But Not Provided

1. Microplate reader (265 nm) and incubator
2. Centrifuge and microcentrifuge tubes
3. High-precision pipette and sterile pipette tips
4. Distilled water
5. Timer
6. Ice
7. Sonicator
8. Mortar

Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Substrate 1 Solution**
  Add 1 ml of Reaction Buffer into the Substrate 1 vial and mix thoroughly to prepare the Substrate 1 Solution. Ensure that the Substrate 1 has completely dissolved prior to use.
- **Substrate 2 Solution**
  Add 1 ml of Reaction Buffer into the Substrate 2 vial and mix thoroughly to prepare the Substrate 2 Solution. Ensure that the Substrate 2 has completely dissolved prior to use.

2. Sample

- **Cell and Bacterial samples**
  Count the number of cells in the culture. Collect the cells into a centrifuge tube, and centrifuge to precipitate the cells. Discard the supernatant, and add 1 ml of Assay Buffer for every 5,000,000 cells. Sonicate at 20% power in 3 second bursts with a 10 second rest interval, for 30 bursts in total. Centrifuge at 16,000 × g at 4°C for 20 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.
- **Tissue samples**
  Homogenize 0.1 g of sample in 1 ml of Assay Buffer on ice for 1 hour. Centrifuge at 16,000 × g at 4°C for 20 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

B. Assay Procedure

Bring all reagents to room temperature prior to use.

1. Set the sample and blank wells on the 96 well microplate and record their positions. We recommend setting up each sample in duplicate.
2. Add 160 µl of Reaction Buffer to all wells.
3. Add 10 µl of Substrate 1 Solution to all wells.
4. Add 10 µl of Substrate 2 Solution to all wells.
5. Add 20 µl of distilled water to the blank wells.
6. Add 20 µl of sample to the sample wells.
7. Tap the plate gently to mix. Start the timer, then read and record absorbance at 265 nm after 10 seconds and after 130 seconds.

C. Calculations

One Unit (U) of Dehydroascorbate Reductase activity is defined as the quantity of enzyme required to produce 1 µmol of ascorbate per minute.

Dehydroascorbate Reductase activity per mg of protein:

$$\textbf{Dehydroascorbate Reductase}\ (\mathbf{U/mg}) = \frac{\mathbf{V_{Total}}}{\mathbf{V_{Sample}} \times \mathbf{C_{Protein}} \times \mathbf{T}} \times \frac{\left(\mathbf{OD_{Sample(130s)}} - \mathbf{OD_{Sample(10s)}}\right) - \left(\mathbf{OD_{Blank(130s)}} - \mathbf{OD_{Blank(10s)}}\right)}{\varepsilon \times \mathbf{d}}$$

$$= \frac{\mathbf{0.153}}{\mathbf{C_{Protein}}} \times \left(\left(\mathbf{OD_{Sample(130s)}} - \mathbf{OD_{Sample(10s)}}\right) - \left(\mathbf{OD_{Blank(130s)}} - \mathbf{OD_{Blank(10s)}}\right)\right)$$

Dehydroascorbate Reductase activity per g of sample:

$$\textbf{Dehydroascorbate Reductase}\ (\mathbf{U/g}) = \frac{\mathbf{V_{Total}} \times \mathbf{V_{Assay}}}{\mathbf{V_{Sample}} \times \mathbf{W} \times \mathbf{T}} \times \frac{\left(\mathbf{OD_{Sample(130s)}} - \mathbf{OD_{Sample(10s)}}\right) - \left(\mathbf{OD_{Blank(130s)}} - \mathbf{OD_{Blank(10s)}}\right)}{\varepsilon \times \mathbf{d}}$$

$$= \frac{\mathbf{0.153}}{\mathbf{W}} \times \left(\left(\mathbf{OD_{Sample(130s)}} - \mathbf{OD_{Sample(10s)}}\right) - \left(\mathbf{OD_{Blank(130s)}} - \mathbf{OD_{Blank(10s)}}\right)\right)$$

Dehydroascorbate Reductase activity per 10^4 cells or bacteria:

$$\textbf{Acetolactate Synthase}\ (\mathbf{U/10^4\ cells}) = \frac{\mathbf{V_{Total}} \times \mathbf{V_{Assay}}}{\mathbf{V_{Sample}} \times \mathbf{N} \times \mathbf{T}} \times \frac{\left(\mathbf{OD_{Sample(130s)}} - \mathbf{OD_{Sample(10s)}}\right) - \left(\mathbf{OD_{Blank(130s)}} - \mathbf{OD_{Blank(10s)}}\right)}{\varepsilon \times \mathbf{d}}$$

$$= \frac{\mathbf{0.153}}{\mathbf{N}} \times \left(\left(\mathbf{OD_{Sample(130s)}} - \mathbf{OD_{Sample(10s)}}\right) - \left(\mathbf{OD_{Blank(130s)}} - \mathbf{OD_{Blank(10s)}}\right)\right)$$

where:

$\mathbf{C_{Protein}}$	Concentration of protein (in mg/ml)
$\boldsymbol{\varepsilon}$	Molar extinction coefficient (5.42×10^4 L /mol /cm = 54.2 ml /µmol /cm)
d	Optical path for a 96-well microplate (0.6 cm)
W	Weight of the sample (in g)
N	Number of cells or bacteria ($\times 10^4$)
$\mathbf{V_{Assay}}$	Volume of assay buffer (1 ml)
$\mathbf{V_{Sample}}$	Volume of sample (0.02 ml)
$\mathbf{V_{Total}}$	Total volume of the enzymatic reaction (0.2 ml)
T	Reaction time (2 min)