Dehydroascorbic Acid (DHA) Assay Kit

Catalog No.: abx298815

Size: 100 Assays

Storage: Store the Enzyme in the dark at -20°C and all the other components in the dark at 4°C.

Application: For quantitative detection of Dehydroascorbic acid (DHA) concentrations in tissue homogenates and cell lysates.

Detection Range: 50 µmol/L – 500 µmol/L

Introduction: Dehydroascorbic acid (DHA) is an oxidized form of ascorbic acid (vitamin C) commonly used as a biomarker of oxidative stress in a variety of experimental models. The assay is initiated by reduction of DHA to ascorbic acid in the presence of thiol-containing reducing agents such as Dithiothreitol (DTT). The assay involves the indirect measurement of DHA where the concentration is calculated by subtraction of the measured ascorbic acid concentration from that of total ascorbic acid analyzed after reduction of the dehydroascorbic acid.

Abbexa's Dehydroascorbic (DHA) Assay Kit is a quick, convenient, and sensitive method for measuring and calculating DHA concentrations. The enzyme reacts with DHA to create an absorption maximum at 265 nm. The intensity of the color is proportional to the concentration of DHA, which can then be calculated.

Kit components

1. 96 well microplate
2. Assay Buffer: 4 × 30 ml
3. Enzyme: 1 vial
4. Enzyme Diluent: 18 ml
5. Standard: 1 vial
6. Plate sealer: 3

Materials Required But Not Provided

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

Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Enzyme Working Solution**
  Add 1 ml of Enzyme Diluent into the Enzyme vial and mix thoroughly. Ensure that the Enzyme has completely dissolved. Transfer the entire contents of the Enzyme vial into the Enzyme Diluent vial and mix thoroughly to prepare the Enzyme Working Solution. Prepare immediately before use.

- **Standard Solution**
  Add 10 ml of distilled water into the Standard vial and mix thoroughly. Ensure that the Standard has completely dissolved. Take 250 µl of this solution and add 750 µl of distilled water to prepare the Standard Solution (concentration 500 µmol/L). Unused Standard Solution can be stored in the dark at 4°C.

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 pre-cooled tube, then analyze immediately.

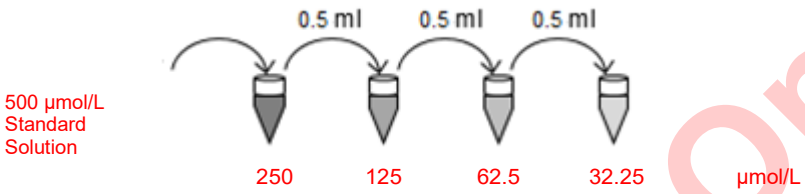
- **Tissue samples**
  Homogenize 0.1 g of sample in 1 ml of Assay Buffer on ice. Centrifuge at 16,000 × g at 4°C for 20 minutes. Transfer the supernatant to a new pre-cooled tube, then analyze immediately.

B. Assay Procedure

Warm all reagents to 37°C prior to use.

If the expected concentration is unknown, a preliminary experiment is recommended to determine if sample dilutions or adjustments to the reaction time are required to bring the measured concentration within the detection range of the kit.

1. Label 4 tubes with 250 µmol/L, 125 µmol/L, 62.5 µmol/L, and 31.25 µmol/L. Aliquot 0.5 ml of distilled water into each tube. Add 0.5 ml of 500 µmol/L Standard Solution to the 1st tube and mix thoroughly. Transfer 0.5 ml from the 1st tube to the 2nd tube and mix thoroughly, and so on.



2. Set the sample and standard wells on the 96 well microplate and record their positions. We recommend setting up each standard and sample in duplicate.
3. Add 20 µl of sample to the sample wells.
4. Add 20 µl of prepared standards to the standard wells.
5. Add 180 µl of Enzyme Working Solution to all wells.
6. Tap the plate gently to mix. Start the timer, then read and record the absorbance at 265 nm after 10 seconds and 130 seconds.

C. Calculations

Dehydroascorbic acid (DHA) concentration per mg of protein:

$$\mathbf{DHA\ (\mu mol/mg)} = \frac{\mathbf{C_{Standard} \times V_{Standard}}}{\mathbf{C_{Protein} \times V_{Sample}}} \times \frac{\mathbf{OD_{Sample(130s)} - OD_{Sample(10s)}}}{\mathbf{OD_{Standard(130s)} - OD_{Standard(10s)}}} = \frac{\mathbf{0.5}}{\mathbf{C_{Protein}}} \times \frac{\mathbf{OD_{Sample(130s)} - OD_{Sample(10s)}}}{\mathbf{OD_{Standard(130s)} - OD_{Sample(10s)}}}$$

Dehydroascorbic acid (DHA) concentration per g of sample:

$$\mathbf{DHA\ (\mu mol/g)} = \frac{\mathbf{C_{Standard} \times V_{Standard} \times V_{Assay}}}{\mathbf{W \times V_{Sample}}} \times \frac{\mathbf{OD_{Sample(130s)} - OD_{Sample(10s)}}}{\mathbf{OD_{Standard(130s)} - OD_{Standard(10s)}}} = \frac{\mathbf{0.5}}{\mathbf{W}} \times \frac{\mathbf{OD_{Sample(130s)} - OD_{Sample(10s)}}}{\mathbf{OD_{Standard(130s)} - OD_{Sample(10s)}}}$$

Dehydroascorbic acid (DHA) concentration per 10^4 cells or bacteria:

$$\mathbf{DHA\ (\mu mol/10^4\ cells)} = \frac{\mathbf{C_{Standard} \times V_{Standard} \times V_{Assay}}}{\mathbf{N \times V_{Sample}}} \times \frac{\mathbf{OD_{Sample(130s)} - OD_{Sample(10s)}}}{\mathbf{OD_{Standard(130s)} - OD_{Standard(10s)}}} = \frac{\mathbf{0.5}}{\mathbf{N}} \times \frac{\mathbf{OD_{Sample(130s)} - OD_{Sample(10s)}}}{\mathbf{OD_{Standard(130s)} - OD_{Sample(10s)}}}$$

where:

$\mathbf{C_{Protein}}$	Concentration of protein (in mg/ml)
$\mathbf{C_{Standard}}$	Concentration of highest standard (500 µmol/L = 0.5 µmol/ml)
$\mathbf{W}$	Weight of the sample (in g)
$\mathbf{N}$	Number of cells or bacteria (× 10^4)
$\mathbf{V_{Assay}}$	Volume of Assay Buffer (1 ml)
$\mathbf{V_{Sample}}$	Volume of sample (0.02 ml)
$\mathbf{V_{Standard}}$	Volume of standard (0.02 ml)