ab65346

Ascorbic Acid Assay Kit

Instructions for Use

For the rapid, sensitive and accurate measurement of Ascorbic Acid in various samples

This product is for research use only and is not intended for diagnostic use.
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1. Overview

Ascorbic Acid (Vitamin C) plays an important role in many biological processes. It is a potent anti-oxidant, anti-inflammatory, anti-viral agent, and an immune stimulant and is present in a wide variety of foods and biological specimens. It is important to be able to monitor ascorbic acid content in these different samples.

Abcam’s Ascorbic Acid Assay Kit provides a rapid, simple, and sensitive means of detecting ascorbic acid in various biological samples. In this assay, our proprietary catalyst oxidizes ascorbic acid to produce a product that interacts with the ascorbic acid probe, generating color and fluorescence. Ascorbic acid can be easily determined by either colorimetric (spectrophotometry at $\lambda = 570$ nm) or fluorometric (Ex/Em = 535/587 nm) methods. The assay can detect 0.01-10 nmol of ascorbic acid per assay in various samples.
2. Protocol Summary

Standard Curve Preparation

Sample Preparation

Add Catalyst

Add Ascorbic Acid Reaction Mix

Measure OD or Fluorescence within 2-3 min
Components and Storage

A. Kit Components

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbate Acid Buffer</td>
<td>25 mL</td>
</tr>
<tr>
<td>Ascorbic Acid Probe (DMSO)</td>
<td>0.2 mL</td>
</tr>
<tr>
<td>Catalyst</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>Ascorbic Acid Enzyme Mix (Lyophilized)</td>
<td>1 vial</td>
</tr>
<tr>
<td>Ascorbic Acid Standard (20 µmol)</td>
<td>1 vial</td>
</tr>
</tbody>
</table>

* Store kit at -20°C, protect from light. Warm Assay Buffer to room temperature before use. Briefly centrifuge all small vials prior to opening.

ASCORBIC PROBE: Ready to use as supplied. Warm to room temperature prior to use to completely melt frozen DMSO, then vortex to ensure uniformity. Store at -20°C, protect from light and moisture. Use within two months.

ASCORBIC ACID ENZYME MIX: Dissolve in 220 µl Ascorbic Acid Assay Buffer. Aliquot and store at -20°C. Use within two months.
ASCORBIC STANDARD: Dissolve in 200 μl of distilled water to generate 100 mM Ascorbic Standard stock solution. Store at -20°C. Use within two months.

CATALYST: Ready to use as supplied

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Fluorescent or colorimetric microplate reader
- 96 well plate
- Orbital shaker
3. Assay Protocol

1. Standard Curve Preparation:
   a. For the colorimetric assay:
      Dilute the standard to 1 mM by adding 10 μl of the 100 mM Ascorbic Acid Standard to 990 μl of distilled water, mix well. Add 0, 2, 4, 6, 8, 10 μl into each well individually. Adjust volume to 120 μl/well with Ascorbic Acid Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of Ascorbic Acid Standard.

   b. For the fluorometric assay:
      Dilute the Ascorbic Acid Standard to 0.01- 0.1 mM with the Ascorbic Acid Assay Buffer (Detection sensitivity is 10 to 100 fold higher for a fluorometric than a colorimetric assay). Follow the procedure for the colorimetric assay.

Note:
Diluted ascorbic acid standard is unstable, use fresh dilution each time.

2. Sample Preparation:
Prepare test samples to a final volume of 120 μl/well with Ascorbic Acid Assay Buffer in a 96-well plate.

We suggest testing several doses of your sample to make sure the readings are within the standard curve range.
Notes:

a) Due to high protein content and other compounds present in plasma and serum we recommend using ab65656 (Ascorbic Acid Assay Kit (Biological Samples)) for plasma and serum samples.

b) Ascorbate is easily oxidized during sample preparation and great care must be exercised to achieve quantitative recovery.

3. Add 100 μl of catalyst to 900 μl of distilled water and vortex well. Add 30 μl of catalyst to each standard and sample well.

4. Ascorbic Acid Reaction Mix: Mix enough reagent for the number of samples and standards to be performed: For each well, prepare a total 50 μl Reaction Mix containing:
   - Ascorbic Acid Assay Buffer  46 μl
   - Ascorbic Acid Probe        2 μl
   - Ascorbic Acid Enzyme Mix   2 μl

    The fluorometric assay is ~10 times more sensitive than the colorimetric assay. Use 0.4 μl of the probe per reaction to decrease the background reading/increase detection sensitivity significantly.

Mix well. Add 50 μl of the Reaction Mix to each well containing the Ascorbic Acid Standard and test samples. Mix well.

Note: Protect from light, Color is developed within 3 min and stable for an hour.
5. Measure $\text{OD}_{570\text{nm}}$ for colorimetric assay or $\text{Ex}/\text{Em} = 535/590$ nm for fluorometric assay in a micro-plate reader.
Data Analysis

Correct background by subtracting the value derived from the zero ascorbic acid standard from all sample readings. The background reading can be significant and must be subtracted from sample readings.

Apply sample readings to the generated standard curve.

Ascorbic Acid concentration can then be calculated:

\[
\text{Concentration} = \frac{\text{As}}{\text{Sv}} \text{ (nmol/μl or μmol/ml or mM)}
\]

Where:
- \(\text{As}\) is ascorbic acid amount from standard curve (nmol).
- \(\text{Sv}\) is the sample volume added in sample wells (μl).

**Ascorbic Acid molecular weight**: 176.12.

Standard Curve performed according to assay protocol.
## 4. Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Reason</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay not working</td>
<td>Assay buffer at wrong temperature</td>
<td>Assay buffer must not be chilled - needs to be at RT</td>
</tr>
<tr>
<td></td>
<td>Protocol step missed</td>
<td>Re-read and follow the protocol exactly</td>
</tr>
<tr>
<td></td>
<td>Plate read at incorrect wavelength</td>
<td>Ensure you are using appropriate reader and filter settings (refer to datasheet)</td>
</tr>
<tr>
<td></td>
<td>Unsuitable microtiter plate for assay</td>
<td>Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells</td>
</tr>
<tr>
<td>Unexpected results</td>
<td>Measured at wrong wavelength</td>
<td>Use appropriate reader and filter settings described in datasheet</td>
</tr>
<tr>
<td></td>
<td>Samples contain impeding substances</td>
<td>Troubleshoot and also consider deproteinizing samples</td>
</tr>
<tr>
<td></td>
<td>Unsuitable sample type</td>
<td>Use recommended samples types as listed on the datasheet</td>
</tr>
<tr>
<td></td>
<td>Sample readings are outside linear range</td>
<td>Concentrate/ dilute samples to be in linear range</td>
</tr>
<tr>
<td>Samples with inconsistent readings</td>
<td>Unsuitable sample type</td>
<td>Refer to datasheet for details about incompatible samples</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>------------------------</td>
<td>----------------------------------------------------------</td>
</tr>
<tr>
<td></td>
<td>Samples prepared in the wrong buffer</td>
<td>Use the assay buffer provided (or refer to datasheet for instructions)</td>
</tr>
<tr>
<td></td>
<td>Samples not deproteinized (if indicated on datasheet)</td>
<td>Use the 10kDa spin column (ab93349)</td>
</tr>
<tr>
<td></td>
<td>Cell/ tissue samples not sufficiently homogenized</td>
<td>Increase sonication time/ number of strokes with the Dounce homogenizer</td>
</tr>
<tr>
<td></td>
<td>Too many freeze-thaw cycles</td>
<td>Aliquot samples to reduce the number of freeze-thaw cycles</td>
</tr>
<tr>
<td></td>
<td>Samples contain impeding substances</td>
<td>Troubleshoot and also consider deproteinizing samples</td>
</tr>
<tr>
<td></td>
<td>Samples are too old or incorrectly stored</td>
<td>Use freshly made samples and store at recommended temperature until use</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lower/ Higher readings in samples and standards</th>
<th>Not fully thawed kit components</th>
<th>Wait for components to thaw completely and gently mix prior use</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Out-of-date kit or incorrectly stored reagents</td>
<td>Always check expiry date and store kit components as recommended on the datasheet</td>
</tr>
<tr>
<td></td>
<td>Reagents sitting for extended periods on ice</td>
<td>Try to prepare a fresh reaction mix prior to each use</td>
</tr>
<tr>
<td></td>
<td>Incorrect incubation time/ temperature</td>
<td>Refer to datasheet for recommended incubation time and/ or temperature</td>
</tr>
<tr>
<td></td>
<td>Incorrect amounts used</td>
<td>Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)</td>
</tr>
<tr>
<td>Problem</td>
<td>Reason</td>
<td>Solution</td>
</tr>
<tr>
<td>---------------------------------------------</td>
<td>--------------------------------------------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Standard curve is not linear</td>
<td>Not fully thawed kit components</td>
<td>Wait for components to thaw completely and gently mix prior use</td>
</tr>
<tr>
<td>Pipetting errors when setting up the standard curve</td>
<td>Try not to pipette too small volumes</td>
<td></td>
</tr>
<tr>
<td>Incorrect pipetting when preparing the reaction mix</td>
<td>Always prepare a master mix</td>
<td></td>
</tr>
<tr>
<td>Air bubbles in wells</td>
<td>Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates</td>
<td></td>
</tr>
<tr>
<td>Concentration of standard stock incorrect</td>
<td>Recheck datasheet for recommended concentrations of standard stocks</td>
<td></td>
</tr>
<tr>
<td>Errors in standard curve calculations</td>
<td>Refer to datasheet and re-check the calculations</td>
<td></td>
</tr>
<tr>
<td>Use of other reagents than those provided with the kit</td>
<td>Use fresh components from the same kit</td>
<td></td>
</tr>
</tbody>
</table>

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select “contact us” on www.abcam.com for the phone number for your region).
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