

## Product Information

### Ascorbic Acid Colorimetric Assay Kit II (FRASC)

#### I. Kit Contents:

Components	K2214-100 100 assays	Cap Color	Part Number
FRASC Buffer	25 ml	WM	K2214-C-1
Ascorbic Acid Probe	1 ml	Red	K2214-C-2
FeCl <sub>3</sub> solution	1 ml	Brown	K2214-C-3
Ascorbate Oxidase (lyophilized)	1 vial	Green	K2214-C-4
Ascorbic Acid Standard (20 μmole)	1 vial	Yellow	A8158

#### II. Introduction:

Ascorbic Acid (Vitamin C) is a reductone and plays important roles in many biological processes. Ascorbic Acid is present in a wide variety of biological specimens and foods. Ascorbic Acid is a potent anti-oxidant, anti-viral and anti-inflammatory agent, and acts as an immune stimulant. Measurement of ascorbic acid content in different samples is important. Because of the presence of many other antioxidants in biological samples such as serum, most ascorbic acid assays show strong interference.

The Ascorbic Acid Colorimetric Assay Kit II (FRASC) provides a sensitive, fast and convenient way for detection of ascorbic acid levels in various samples (serum and other body fluids, tissue and cell extracts, growth media and food products) based on colorimetric method. In the assay, Fe<sup>3+</sup> is reduced to Fe<sup>2+</sup> by any antioxidants present. The Fe<sup>2+</sup> iron is chelated with a colorimetric probe to generate a product with a strong absorbance band between 545 - 600 nm. The kit can detect 0.2 to 20 nmol ascorbic acid in various samples.

#### III. Storage and Handling:

Store kit at –20°C, protect from light. Warm FRASC Buffer to room temperature before use. Briefly centrifuge all small vials prior to opening. Make sure the plate reader is turned on and ready to use.

Reagent Preparation: FeCl<sub>3</sub>: Ready to use as supplied. Stable for two months at room temperature.

Ascorbate Probe: Ready to use as supplied. Stable for two months at room temperature.

Ascorbic Oxidase: Dissolve in 500 μl distilled water. Aliquot and store at –20°C. Use within two months.

Ascorbic Standard: Dissolve in 200 μl of distilled water to generate 100 nmol/μl of Ascorbic Standard stock solution. Store at –20°C. Use within two months.

#### IV. Ascorbic Acid Assay Protocol:

1. Standard Curve Preparations: Dilute the standard to 1 nmol/μl by adding 10 μl of the 100 nmol/μl 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 100 μl/well with distilled water to generate 0, 2, 4, 6, 8, 10 nmol/well of Ascorbic Acid Standard.

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

2. Sample Preparation: Add up to 100 μl/well of sample to a paired set of wells in a 96-well plate. One well of the pair is the total antioxidant present; the 2nd well is the background (ascorbate depleted). If the sample is less than 100 μl, make up the volume with distilled water. Test several doses of your sample to ensure readings are within the linear range of the assay (0 - 2.5 OD). Note: Cells and tissues can be homogenized in dH<sub>2</sub>O.

3. Add 10 μl distilled water to the total antioxidant well; 10 μl of Ascorbate Oxidase to the background well.

4. Incubate the plate at room temperature for 15 minutes to deplete all ascorbate.

5. Ascorbic Acid Reaction Mix: Mix enough reagent for the number of samples and standards to be performed: For each well, prepare a total 100  $\mu$ l Reaction Mix containing:

FRASC Buffer            80  $\mu$ l

Ascorbic Acid Probe    10  $\mu$ l

FeCl<sub>3</sub>                    10  $\mu$ l

6. Add 100  $\mu$ l of the Reaction Mix to each well containing the Ascorbic Acid Standard and test samples. Mix well.

7. Read within 2 - 3 min. Under these experimental conditions, ascorbate reacts almost instantaneously with the reagent while other antioxidants react much more slowly, so the longer the wait time the higher the background will be.

8. Measure OD at 593 nm. Wavelengths between 545 and 600 nm are acceptable as they will give 90 % of the maximum absorbance. 9. Subtract the value of the Background wells (containing Ascorbate Oxidase) from the wells with total antioxidants present. The difference is OD due to ascorbic acid. Determine the nmoles of ascorbate from the standard curve.

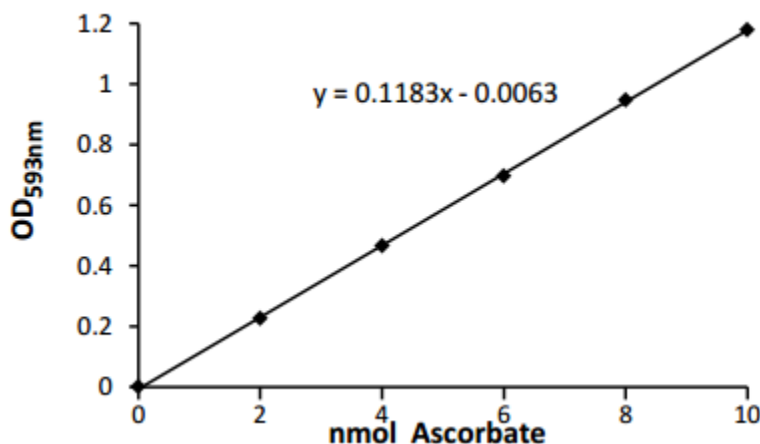
$$C = \text{ascorbate concentration in sample} = (A_t - A_b) / (\text{slope of the standard curve}) / V = \text{nmol/ml} = \mu\text{M}$$

Where:  $A_t$  is the absorbance of the total antioxidant well.

$A_b$  is the absorbance of the background well with ascorbate Oxidase .

Slope is absorbance of 10 nmol standard – 0 nmol standard/10 nmol.

V is the sample volume added into the reaction well (in ml).



### General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	<ul style="list-style-type: none"> <li>• Use of a different buffer</li> <li>• Omission of a step in the protocol</li> <li>• Plate read at incorrect wavelength</li> <li>• Use of a different 96-well plate</li> </ul>	<ul style="list-style-type: none"> <li>• Assay buffer must be at room temperature</li> <li>• Refer and follow the data sheet precisely</li> <li>• Check the wavelength in the data sheet and the filter settings of the instrument</li> <li>• Fluorescence: Black plates ; Luminescence: White plates; Colorimeters: Clear plates</li> </ul>
Samples with	<ul style="list-style-type: none"> <li>• Use of an incompatible sample type</li> </ul>	<ul style="list-style-type: none"> <li>• Refer data sheet for details about incompatible samples</li> </ul>

erratic readings	<ul style="list-style-type: none"> <li>• Samples prepared in a different buffer</li> <li>• Samples were not deproteinized (if indicated in datasheet)</li> <li>• Cell/ tissue samples were not completely homogenized</li> <li>• Samples used after multiple free-thaw cycles</li> <li>• Presence of interfering substance in the sample</li> <li>• Use of old or inappropriately stored samples</li> </ul>	<ul style="list-style-type: none"> <li>• Use the assay buffer provided in the kit or refer data sheet for instructions</li> <li>• Use the 10 kDa spin cut-off filter or PCA precipitation as indicated</li> <li>• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope</li> <li>• Aliquot and freeze samples if needed to use multiple times</li> <li>• Troubleshoot if needed, deproteinize samples</li> <li>• Use fresh samples or store at correct temperatures till use</li> </ul>
Lower/ Higher readings in Samples and Standards	<ul style="list-style-type: none"> <li>• Improperly thawed components</li> <li>• Use of expired kit or improperly stored reagents</li> <li>• Allowing the reagents to sit for extended times on ice</li> <li>• Incorrect incubation times or temperatures</li> <li>• Incorrect volumes used</li> </ul>	<ul style="list-style-type: none"> <li>• Thaw all components completely and mix gently before use</li> <li>• Always check the expiry date and store the components appropriately</li> <li>• Always thaw and prepare fresh reaction mix before use</li> <li>• Refer data sheet &amp; verify correct incubation times and temperatures</li> <li>• Use calibrated pipettes and aliquot correctly</li> </ul>
Readings do not follow a linear pattern for Standard curve	<ul style="list-style-type: none"> <li>• Use of partially thawed components</li> <li>• Pipetting errors in the standard</li> <li>• Pipetting errors in the reaction mix</li> <li>• Air bubbles formed in well</li> <li>• Standard stock is at an incorrect concentration</li> <li>• Calculation errors</li> <li>• Substituting reagents from older kits/ lots</li> </ul>	<ul style="list-style-type: none"> <li>• Thaw and resuspend all components before preparing the reaction mix</li> <li>• Avoid pipetting small volumes</li> <li>• Prepare a master reaction mix whenever possible</li> <li>• Pipette gently against the wall of the tubes</li> <li>• Always refer the dilutions in the data sheet</li> <li>• Recheck calculations after referring the data sheet</li> <li>• Use fresh components from the same kit</li> </ul>
Unanticipated results	<ul style="list-style-type: none"> <li>• Measured at incorrect wavelength</li> <li>• Samples contain interfering substances</li> <li>• Use of incompatible sample type</li> <li>• Sample readings above/below the linear range</li> </ul>	<ul style="list-style-type: none"> <li>• Check the equipment and the filter setting</li> <li>• Troubleshoot if it interferes with the kit</li> <li>• Refer data sheet to check if sample is compatible with the kit or optimization is needed</li> <li>• Concentrate/ Dilute sample so as to be in the linear range</li> </ul>
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		

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## Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit <http://www.apexbt.com/> or contact our technical team.



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