Ascorbic Acid Colorimetric Assay Kit II (FRASC)

(Catalog #K671-100; 100 assays; Store at -20°C)

I. Introduction:

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 biological specimens. Due to the presence of a variety of other antioxidants in biological samples such as serum, most ascorbic acid assays show strong interference. BioVision's FRASC Assay Kit provides a rapid, simple, and sensitive means of detecting ascorbic acid in biological samples such as serum and other body fluids, tissue and cell extracts, growth media and food products. In this assay, Fe^{+3} is reduced to Fe^{+2} by any antioxidants present. The ferrous iron is chelated with a colorimetric probe to produce a product with a strong absorbance band which can be monitored between 545 - 600 nm. The addition of ascorbate oxidase to parallel samples removes any ascorbate present leaving a background value which is subtracted from the total to give ascorbate content. The assay can detect 0.2 to 20 nmol of ascorbic acid in various samples.

II. Kit Contents:

Components	K671-100	Cap Code	Part Number
FRASC Buffer	25 ml	WM	K671-100-1
Ascorbic Acid Probe	1 ml	Red	K671-100-2
$FeCl_3$ solution	1 ml	Brown	K671-100-3
Ascorbate Oxidase (lyophilized)	1 vial	Green	K671-100-4
Ascorbic Acid Standard (20 µmole)	1vial	Yellow	K671-100-5

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:

$FeCl_3$: 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:

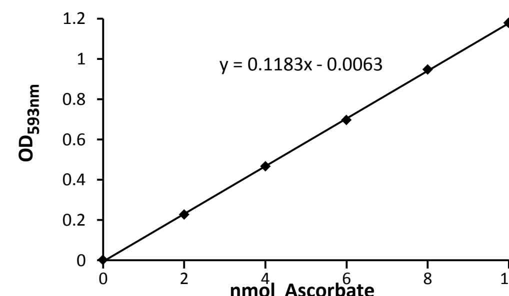
1. **Standard Curve Preparations:**
Dilute the standard to 1 nmol/µl by adding 10 µl of the 100 nmol/µl Ascorbic Acid Standard to 990 µl of distilled water, mix well. Add 0, 2, 4, 6, 8, 10 µl into each well individually. Adjust volume to 100 µl/well with distilled water to generate 0, 2, 4, 6, 8, 10 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_2O.
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 µl Reaction Mix containing:

80 µl	FRASC Buffer
10 µl	Ascorbic Acid Probe
10 µl	$FeCl_3$

6. Add 100 µl 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 = ascorbate concentration in sample
= (At – Ab)/(slope of the standard curve)/V = nmol/ml = µM

Where: **At** is the absorbance of the total antioxidant well
Ab 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)



RELATED PRODUCTS:

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Cell Proliferation & Senescence Kits
Cholesterol, LDL/HDL Assay Kits
Ethanol and Uric Acid Assay Kit
NAD(P)H Assay Kit

FOR RESEARCH USE ONLY! Not to be used on humans.

GENERAL TROUBLESHOOTING GUIDE:

Problems	**Cause**	**Solution**
Assay not working	• Use of ice-cold assay buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Samples were not deproteinized (if indicated in datasheet) • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use the 10 kDa spin cut-off filter or PCA precipitation as indicated • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer datasheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	• Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		