Ferric Reducing Ascorbate (FRASC) Assay Kit

(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. amsbio'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 Enzyme Mix: 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).

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 minutes. 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 O.D. 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: $V = \frac{\text{Slope}}{\text{V}}$

At is the absorbance of the total antioxidant well
Ab is the absorbance of the background well with ascorbate oxidase
is absorbance of 10 nmol standard – 0 nmol standard/10 nmol
is the sample volume added into the reaction well (in ml)