K-*ASSAY*®

Vitamin C

For the Quantitative Determination of Vitamin C

Cat. No. KT-75000

INTENDED USE

For the quantitative determination of vitamin C in serum, plasma, and other samples. FOR RESEARCH USE ONLY IN THE U.S. NOT FOR USE IN DIAGNOSTIC PROCEDURES IN THE U.S.

INTRODUCTION AND SUMMARY

Vitamin C (L-Ascorbic acid) is a water-soluble vitamin with strong reduction ability and is important as a coenzyme of the internal hydroxylation reactions such as with collagen composition. Vitamin C is divided into reduced form (Ascorbic Acid (AsA)) and oxidized form (Dehydroascorbic Acid (DHAsA)). This Vitamin C assay measures total Vitamin C (AsA and DHAsA).

Vitamin C is a six-carbon lactone that is synthesized from glucose in the liver of most mammalian species, but not by humans. Therefore, humans must obtain ascorbate in their diet in order to survive. In humans, ascorbate acts as an electron donor for eight different enzymes. It also serves as an antioxidant and may be beneficial for reducing the risk of developing chronic diseases such as cancer, cardiovascular disease, and cataracts.

PRINCIPLE OF TEST

The **K-*ASSAY*®** Vitamin C assay quantifies the vitamin C in serum or plasma based on an enzymic method.

Ascorbate Oxidase catalyzes the oxidation of L-Ascorbate (vitamin C) to generate hydrogen peroxide. Peroxidase catalyzes the hydrogen peroxide and the unique chromogen to form blue dye, which enables high sensitive measurement. The reduction effect of Vitamin C has no effect on this reagent.

The **K-*ASSAY*®** Vitamin C assay can be run using a two-reagent clinical chemistry analyzer. The **K-*ASSAY*®** Vitamin C Calibrator is used in conjunction with a 0-point saline to prepare a 2-point calibration. This calibration is then used for quantifying the levels of vitamin C present in serum or plasma samples.

KIT COMPOSITION

Reagents (Liquid Stable)

R1: Chromogen Reagent, pH 5.5

R2: Enzyme Reagent, pH 5.5
Ascorbic Acid Oxidase
Peroxidase

WARNINGS AND PRECAUTIONS

For Research Use Only in the U.S. Not For Use in Diagnostic Procedures in the U.S.

Not to be used internally in humans or animals. Normal precautions exercised in handling laboratory reagents should be followed.

Do not mix or use reagents from one test kit with those from a different lot number.

Do not use reagents past their expiration date stated on each reagent container label.

Do not pipette by mouth. Avoid ingestion and contact with skin.

The Sample Stabilizer contains metaphosphoric acid, a corrosive material. It may cause skin burns or eye damage and is also harmful to aquatic life. Wear protective clothing including glasses and gloves and handle with care.

REAGENT PREPARATION

The reagents and sample stabilizer must be thawed and warmed to room temperature prior to use. Gentle mixing prior to use is recommended.

STORAGE AND HANDLING

The R-1 and R-2 reagents should be stored frozen (-20°C or below). Unopened reagents can be used for 36 months from date of manufacture, as indicated by the expiration date on package and bottle labels.

Thawed R-1 and R-2 reagents can be used for 24 hours after opening if kept at 2-8°C.

Sample Stabilizer may be kept at room temperature after thawing and is stable after opening until the expiration date on package and bottle labels.

REAGENT STABILITY

Discard reagents if they become contaminated. Evidence of obvious precipitation in reagent 2 (R-2) solution is cause to discard.

INSTRUMENT

Measurement of absorbance is to be made with an instrument able to accurately read absorbance at 660 nm with an optional secondary wavelength of 700nm.

Refer to the instrument manual from the manufacturer regarding the following:

a) Use or function
b) Installation procedures and requirements
c) Principles of operation
d) Performance characteristics, operating instructions
e) Calibration procedures including materials and / or equipment to be used
f) Operational precautions, limitations, and hazards
g) Service and maintenance information

SPECIMEN COLLECTION AND PREPARATION

It is recommended that specimen collection be carried out in accordance with CLSI document M29-A3. No method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood samples should be considered potentially infectious.

For blood samples, sodium-heparin or sodium-lithium collection tubes should be used. EDTA collection tubes are not recommended.

Samples should be tested immediately after collection due to the rapid breakdown of vitamin C.

Otherwise, samples should be treated right away at a 1:1 ratio with **K-*ASSAY*®** Vitamin C Sample Stabilizer and stored at -20°C or below. Treated samples should be used within 2 weeks after collection.

AUTOMATED ANALYZER APPLICATION

Suitable for two-reagent automated analyzers that use a two-point calibration method.

PROCEDURE

Materials Supplied

KT-75000 Vitamin C

Reagent 1 (R-1) Chromogen Reagent	3 x 10 mL
Reagent 2 (R-2) Enzyme Reagent	3 x 5 mL
Sample Stabilizer	2 x 12.5 mL

Materials Required But Not Supplied

Calibrators: **K-*ASSAY*®** Vitamin C Calibrator,
Cat. No. KT-75001

Isotonic Saline

Two Reagent Clinical Chemistry Analyzer:
Capable of accurate absorbance readings at 660 nm
Capable of accurately dispensing the required volumes
Capable of maintaining 37°C

Assay Procedure

Note: Allow all reagents and specimens to warm to room temperature (18-25˚C). Mix all reagents gently before using.

An example of automated application:

Sample 5.0 µL
↓
• ← R1 (Chromogen Reagent) 300 µL
↓ 37 °C, 5 minutes
• ← R2 (Enzyme Reagent) 150 µL
↓ 37 °C, 5 minutes
2 Point Endpoint at 660 nm main,700 nm sub

Automated Method (Example)

Chemistry Parameters for Automatic Analyzer

INSTRUMENT	Roche / Hitachi 911
TEMPERATURE	37°C
TEST	(VitC)
ASSAY CODE	(2 POINT END)(10) : (15) (31) (0) (0)
WAVELENGTH	(700) (660)
SAMPLE VOLUME	(5.0) (0.0) (0)
R-1 VOLUME (R1)	(300) (0)
R-2 VOLUME (R3)	(150) (0)
ABS. LIMIT (SLOPE)	(32000) (32000) (INCREASE)
PROZONE LIMIT	(-32000) (-32000) (LOWER)
CALIB. TYPE	(LINEAR)
POINT	(2)
SPAN POINT	(2)
SD LIMIT	(999)
DUPLICATE LIMIT	(10000)
SENSITIVITY LIMIT	(0)
S1ABS RANGE	(-32000) (32000)
INSTRUMENT FACTOR	a = (1.0) b = (0.0)
UNIT	(mg/dL)
STD.(1) Conc.-POS.	(*1) - (1)
STD.(2) Conc.-POS.	(*2) - (2)

Use isotonic saline as calibrator point 1.

Use Vitamin C Calibrator as calibrator point 2.

*1-2: Input concentration of calibrators.

Parameters for other automated analyzers are available.

CALIBRATION

It is recommended that vitamin C levels be determined using a 2-point calibration curve prepared using a saline blank and the **K-*ASSAY*®** Vitamin C Calibrator. Due to the instability of vitamin C, it is recommended that the assay be calibrated each day when used.

QUALITY CONTROL

Normal and abnormal controls of known concentration should be included in each assay performed. These controls should fall within the ranges established by each

lab for the particular lot of controls. The validity of the assay is in question if the value for the controls generated by the assay's calibration curve does not fall within the stated range. Recalibrate if the value determined for the controls falls outside the established recovery range.

RESULTS / CALCULATIONS

Vitamin C levels are determined by comparison versus the prepared calibration curve.

LIMITATIONS OF PROCEDURE

The measuring range for vitamin C is between 0.05 and 5.00 mg/dL. Samples with very high vitamin C concentrations should be diluted 1 part sample with 4 part isotonic saline. Multiply results by 5 to compensate for the dilution.

PERFORMANCE

Specificity

When a sample with a known value is assayed, the result is within ±10% of the assigned value.

Precision

The precision for the **K-*ASSAY*®** Vitamin C assay was determined using packaged reagents, in-house samples, and a Hitachi 911 chemistry analyzer.

Precision Assay: Within Run

Sample I	Sample II	Sample III
N = 20	N = 20	N = 20
Mean = 0.20	Mean = 1.01	Mean = 4.03
SD = 0.007	SD = 0.009	SD = 0.022
CV = 3.72%	CV = 0.90%	CV = 0.55%

(Concentrations in mg/dL)

Precision Assay: Between Runs

Sample I	Sample II	Sample III
N = 25	N = 25	N = 25
Mean = 0.21	Mean = 1.02	Mean = 4.00
SD = 0.010	SD = 0.08	SD = 0.022
CV = 4.60%	CV = 0.76%	CV = 0.55%

(Concentrations in mg/dL)

Accuracy / Correlation

A comparison of the **K-*ASSAY*®** Vitamin C and an HPLC vitamin C assay was performed with the following results:

y = 0.9734x + 0.0249
r = 0.9958
n = 16
x = HPLC vitamin C assay
y = **K-*ASSAY*®** Vitamin C Assay

x min	= 0.17 mg/dL	y min	= 0.12 mg/dL
max	= 2.38 mg/dL	max	= 2.35 mg/dL
mean	= 1.27 mg/dL	mean	= 1.26 mg/dL

Assay Range

0.05 - 5.00 mg/dL

INTERFERENCE

Bilirubin F and C	No interference up to 32 mg/dL
Hemoglobin	No interference up to 200 mg/dL
Chyle, Intrafat (Turbidity)	No interference up to 2,000 FTU

LABELING SYMBOLS

LOT	Lot Number
RGT	Reagent
	Expiration or “Use By” Date
REF	Catalog Number
-20 °C	Temperature Limitation. Store at -20 degrees C
d	Corrosive
	Manufacturer
	Consult Package Insert for Instructions for Use
EC REP	Authorized Representative in the European Community

EU AUTHORIZED REPRESENTATIVE

CE

EC REP

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