K-*ASSAY*®

Cystatin C

For the Quantitative Determination of Human Cystatin C in Serum or Plasma

Cat. No. KAI-074

INTENDED USE

For the quantitative determination of human cystatin C in serum, EDTA plasma, or lithium heparin plasma by immunoturbidimetric assay. Cystatin C measurements are used as an aid in the diagnosis and treatment of renal diseases. FOR *IN VITRO* DIAGNOSTIC USE.

INTRODUCTION AND SUMMARY

Cystatin C is a small, 13.4 kDa, non-glycosylated basic protein belonging to the cystatin super-family of cysteine protease inhibitors. Cystatin C is produced by virtually all nucleated cells, and is present in all investigated body fluids. The production rate is constant and is unaffected by inflammatory processes, gender, age, and muscle mass.[1] In normal kidneys, cystatin C is almost freely filtered through the glomerular membrane and then nearly completely reabsorbed and degraded by proximal tubular cells. Therefore, the plasma concentration of cystatin C is almost exclusively determined by the glomerular filtration rate (GFR), making cystatin C an excellent indicator of GFR function. Numerous studies and a meta-analysis incorporating 4,492 subject samples have shown that serum cystatin C is superior to serum creatinine as a marker for GFR function.[2]

PRINCIPLE OF TEST

The **K-*ASSAY*®** Cystatin C quantifies the cystatin C in the patient's serum or plasma based on immunoturbidimetric assay.

The cystatin C reagent contains a suspension of latex particles coated with purified goat anti-human cystatin C polyclonal antibodies. A sample is mixed with this suspension. The resulting immune complexes are measured by turbidimetry. The signal generated is correlated with the concentration of cystatin C in the sample. By interpolation on a standard curve, the concentration of cystatin C in the sample is calculated.

The **K-*ASSAY*®** Cystatin C assay can be run using a two-reagent clinical chemistry analyzer. Six calibrators are provided in the **K-*ASSAY*®** Cystatin C Calibrator. These calibrators are used to prepare a calibration curve for quantifying the levels of cystatin C present in the patient's serum or plasma sample.

KIT COMPOSITION

Reagents (Liquid Stable)

R1: Buffer Reagent, pH 7.5
HEPES (50 mM)

R2: Latex Suspension, pH 6.0
Latex particles coated with goat anti-human cystatin C antibodies (0.11% w/v)
MES (25 mM)

WARNINGS AND PRECAUTIONS

For *In Vitro* Diagnostic Use Only. ℞ only.

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.

Reagents in this kit contain < 0.1% w/v sodium azide as a preservative. Sodium azide may form explosive compounds in metal drain lines. When disposing of reagents through plumbing fixtures, flush with copious amounts of water. For further information, refer to "Decontamination of Laboratory Sink Drains to Remove Azide Salts," in the Manual Guide-Safety Management No. CDC-22 issued by the Centers for Disease Control, Atlanta, Georgia.

REAGENT PREPARATION

Reagents are ready to use and do not require reconstitution.

STORAGE AND HANDLING

All reagents should be stored refrigerated (2-8°C). Return all reagents to 2-8°C promptly after use. Unopened reagents can be used for up to 18 months from the date of manufacture, as indicated by the expiration date on package and bottle labels.

REAGENT STABILITY

Opened reagents can be used for one month if stored at 2-8°C. 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 570 nm. 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.

Serum or plasma (EDTA or lithium heparin) test samples must be collected in the manner routinely used for clinical laboratory tests. Freshly drawn serum is preferred and should be used within the day of collection. Samples may also be stored refrigerated (2-8°C) for five days or at -30°C for up to 1 year. Avoid excessive freeze/thaw of specimens.[3,4]

Use plastic tubes for storing the samples, do not use glass.

AUTOMATED ANALYZER APPLICATION

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

PROCEDURE

Materials Supplied

Reagent 1 (R-1) Buffer Reagent	2 x 10 mL
Reagent 2 (R-2) Latex Suspension	2 x 10 mL

Materials Required But Not Supplied

Calibrators: **K-*ASSAY*®** Cystatin C Calibrator, Cat. No. KAI-099C (6 calibrators containing known amounts of human cystatin C).

Two-Reagent Clinical Chemistry Analyzer:
Capable of accurate absorbance readings at 570 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. Mix all reagents gently before using.

An example of automated application (Hitachi 917):

Sample 3.0 µL
↓
• ←R1 (Buffer Reagent) 120 µL
↓ 37 °C, 5 min.
• ←R2 (Latex Suspension) 120 µL
↓ 37 °C, 5 min.
2-point endpoint, 570 /800 nm

Automated Method (Example)

Chemistry Parameters for Automatic Analyzer

INSTRUMENT	Roche / Hitachi 917
TEMPERATURE	37°C
TEST	(CysC)
ASSAY CODE	(2 POINT END)(10) (18)(28)(0)(0)
WAVELENGTH	(800) (570)
SAMPLE VOLUME	(3.0) (0.0) (0)
REAGENT VOL (R1)	(120) (0)
REAGENT VOL (R2)	(0) (0)
REAGENT VOL (R3)	(120) (0)
REAGENT VOL (R4)	(0) (0)
ABS. LIMIT (SLOPE)	(32000) (INCREASE)
PROZONE LIMIT	(-32000)(34)(LOWER)
CALIB. TYPE	(SPLINE)
POINT	(6)
SPAN POINT	(6)
SD LIMIT	(999)
DUPLICATE LIMIT	(10000)
SENSITIVITY LIMIT	(0)
S1ABS RANGE	(-32000) (32000)
INSTRUMENT FACTOR	a=(1.0) b=(0.0)
UNIT	(mg/L)
STD.(1) Conc.-POS.	(*1) - (1)
STD.(2) Conc.-POS.	(*2) - (2)
STD.(3) Conc.-POS.	(*3) - (3)
STD.(4) Conc.-POS.	(*4) - (4)
STD.(5) Conc.-POS.	(*5) - (5)
STD.(6) Conc.-POS.	(*6) - (6)

*1-6: Input concentration of calibrators.

Parameters for other automated analyzers are available.

CALIBRATION

It is recommended that cystatin C levels be determined using a multi-point calibration curve prepared using the **K-*ASSAY*®** Cystatin C Calibrator. On the Roche / Hitachi 917, calibration curves were found to be stable for up to one month. However, it is recommended that the user determine calibration curve frequency as this depends on the instrument and type/number of other assays being performed.

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

Cystatin C levels are determined using the prepared calibration curve.

LIMITATIONS OF PROCEDURE

The measuring range for cystatin C is between 0.40 and 8.00 mg/L (0.34 - 6.80 mg/L ERM-DA471/IFCC Standardized). Grossly lipemic samples and samples with very high triglyceride concentrations should be diluted 1 part sample with 4 part isotonic saline or filtered to decrease nonspecific light scattering. Multiply results by 5 to compensate for the dilution.

If the cystatin C concentration of a patient sample is greater than 8.00 mg/L (6.80 mg/L ERM-DA471/IFCC Standardized), dilute 1 part sample with 3 parts isotonic saline and reassay. Multiply results by 4 to compensate for the dilution.

PERFORMANCE

Precision

The precision for the **K-*ASSAY*®** Cystatin C assay was determined using packaged reagents, control material, and a Roche / Hitachi 917 analyzer according to the CLSI EP5-A2 guideline.

	Sample			
	1	2	3	4
N	80	80	80	80
Mean (mg/L)	0.511	0.968	1.999	4.389
Within Run S.D.	0.006	0.007	0.013	0.030
Within Run C.V. %	1.094	0.712	0.640	0.690
Between Run S.D.	0.005	0.024	0.019	0.079
Between Run C.V. %	1.066	2.496	0.960	1.811
Between Day S.D.	0.005	0.017	0.014	0.023
Between Day C.V. %	0.928	1.776	0.707	0.525
Total S.D.	0.007	0.024	0.027	0.088
Total C.V. %	1.421	2.462	1.353	2.008

Accuracy / Correlation

Testing was performed on a Roche / Hitachi 917 analyzer according to the CLSI EP9-A2 guideline. A comparison of the **K-*ASSAY*®** Cystatin C and another company's cystatin C assay was performed with the following results:

y = 1.0093x + 0.411
r = 0.9983
n = 50
x = another company's Cystatin C assay
y = **K-*ASSAY*®** Cystatin C Assay

x min	=	0.41	
max	=	7.43	
mean	=	2.650	
y min	=	0.40	
max	=	7.68	
mean	=	2.633	

Linearity

Testing was performed on a Roche / Hitachi 917 analyzer according to the CLSI EP6-A guideline on diluted samples and the CLSI EP17-A guideline with the following results.

Linearity: 0.06 - 8.00 mg/L (0.05 - 6.80 mg/L*)

Limit of Blank (LoB) = 0.012 mg/L (0.010 mg/L*)
Limit of Detection (LoD) = 0.024 mg/L (0.020 mg/L*)

(* ERM-DA471/IFCC Standardized)

INTERFERENCE

Testing was performed on a Roche / Hitachi 917 analyzer according to the CLSI EP7-A2 guideline with the following results.

Bilirubin, Conjugated	No interference up to 60 mg/dL
Bilirubin, Unconjugated	No interference up to 60 mg/dL
Hemoglobin	No interference up to 900 mg/dL
Lipemia	No interference up to 1,100 mg/dL
Rheumatoid Factor	No interference up to 1,000 IU/L
Triglycerides	No interference up to 1,500 mg/dL

MEASURING RANGE

Measuring Range: 0.40 - 8.00 mg/L (0.34 - 6.80 mg/L*)

(* ERM-DA471/IFCC Standardized)

EXPECTED VALUE

The expected value as per the literature is between 0.5 and 1.0 mg/L.[5] Due to population differences, each laboratory should establish its own expected values using this kit.

REFERENCES

1. Grubb, A.O. "Cystatin C – properties and use as diagnostic marker." Adv Clin Chem 35: 63-99, 2000.
2. Dharnidharka, V.R. and Kwon C. and Stevens, G. "Serum cystatin C is superior to serum creatitine as a marker of kidney function: A meta-analysis." Am J Kidney Dis 40: 221-226, 2002.
3. Clin. Chem 1994, 40(10), 1921-6.
4. Clin. Chem 1997, 43(6), 1016-22.
5. Tietz, N.W. Clinical Guide to Laboratory Tests, 4th ed., 2005.

LABELING SYMBOLS

REF	Catalog Number
	Expiration or "Use By" Date
LOT	Lot Number
	Consult Package Insert for Instructions for Use
IVD	For *In Vitro* Diagnostic Use
CE	CE Mark Registered
℞	For Prescription Use Only
2°C 8°C	Temperature Limitation. Store between 2 and 8 degrees C
	Manufacturer
EC REP	Authorized Representative in the European Community

EU AUTHORIZED REPRESENTATIVE

CE

EC REP

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