K-*ASSAY*®

CRP (3)

For the Quantitative Determination of C-Reactive Protein (CRP) in Serum and Plasma

Cat. No. KAI-082

INTENDED USE

K-*ASSAY*® CRP (3) is intended to be used as a high-sensitive assay for the quantitative determination of CRP in serum and plasma by immunoturbidimetric assay. Measurement of C-Reactive Protein aids in the detection and evaluation of tissue injury, inflammatory disorders, and related diseases. FOR *IN VITRO* DIAGNOSTIC USE.

INTRODUCTION AND SUMMARY

C-reactive protein (CRP) is described in the literature as an acute phase protein that is involved in the activation of complement, acceleration of phagocytosis, and detoxification of substances released from damaged tissue. CRP is one of the most sensitive indicators of inflammation.

In response to an inflammatory stimulus, a rise in CRP may be detected within 6 hours. CRP is a sensitive, non-specific indicator of acute phase reactants.[1,2,3] The level of CRP in serum is elevated in patients with arthritis and after severe infections such as septic shock.

The **K-*ASSAY*®** CRP (3) is intended for the quantitative determination of human CRP by immunoturbidimetric assay (ITA). ITA methods for quantitative determination of antibody and antigen immunoprecipitation complexes have been described.[4,5,6,7]

PRINCIPLE OF TEST

Latex particles coated with antibody specific to human CRP aggregate in the presence of CRP from the sample forming immune complexes. The immune complexes cause an increase in light scattering, which is proportional to the concentration of CRP in the serum. The light scattering is measured by reading turbidity at 570 nm. The sample CRP concentration is determined versus dilutions of a CRP calibrator of known concentration. The CRP (3) reagent is calibrated with one of three possible CRP (3) calibrator sets. Each calibrator set will enable the assay to provide a different linear range.

KIT COMPOSITION

Reagents (Liquid stable)
KAI-082

R-1: Buffer Reagent 170 mM Glycine buffer solution	1 x 50 mL
R-2: Latex Suspension 0.20% (w/v) solution of latex particles coated with rabbit anti-human CRP antibodies	1 x 50 mL

WARNINGS AND PRECAUTIONS

FOR *IN VITRO* DIAGNOSTIC USE. Rx only.

Not to be used internally in humans or animals. Normal precautions for 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. Mix gently before using.

STORAGE AND STABILITY

All reagents should be stored at 2-8°C and protected from light. Unopened reagents can be used for 18 months from the date of manufacture as indicated on the expiration date on the package and bottle labels. Discard reagents if they become contaminated. Evidence of obvious precipitation in reagent 2 (R-2) solution is cause to discard. Once the reagent vial has been opened, store tightly capped at 2-8°C and use within 1 month.

SPECIMEN COLLECTION AND PREPARATION

Serum or plasma (sodium 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 one week or at -30°C for up to 1 year. Avoid excessive freeze/thaw of specimens.

Very lipemic samples, or frozen samples which become turbid after thawing, should be centrifuged before use. Do not heat sample. Heat inactivation can lead to diminished CRP values. Use undiluted samples for this assay.

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

AUTOMATED ANALYZER APPLICATION

Suitable for two-reagent automated analyzers that can measure a rate reaction at an absorbance at 570 nm. User should validate assay function on instrument. 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

PROCEDURE

Materials Supplied

Reagent 1 (R-1) Buffer Reagent	1 x 50 mL
Reagent 2 (R-2) Latex Suspension	1 x 50 mL

Materials Required But Not Supplied

Multi-point calibrators:

Standard Sensitivity Protocol: **K-*ASSAY*®** CRP (3) Calibrator D, Cat. No. KAI-083C, 5 calibrators; Approx. values: 5, 20, 40, 160, 320 mg/L. (For actual values see Package Insert).

High Sensitivity Protocol: **K-*ASSAY*®** CRP (3) Calibrator E, Cat. No. KAI-084C, 5 calibrators; Approx. values: 2.5, 10, 20, 80, 160 mg/L. (For actual values see Package Insert).

Wide Sensitivity Protocol: **K-*ASSAY*®** CRP (3) Calibrator F, Cat. No. KAI-086C, 5 calibrators; Approx. values: 10, 40, 160, 320, 480 mg/L. (For actual values see Package Insert).

Automated chemistry analyzer: capable of accurate absorbance readings at 570 nm with appropriate cuvettes and calculating rate assays.

Isotonic saline for 0 mg/L calibrator

Pipettes: capable of accurately dispensing the required volumes

Test Tubes: glass or plastic

Assay Procedure

An example of standard protocol automated application:

Sample		3.0 µL
↓		
• ←	R-1 (Buffer Reagent)	150 µL
↓	37°C, 5 min	
• ←	R-2 (Latex Suspension)	150 µL
↓	37°C, 10 min	
Measure 2 point end, 570/800 nm		

Note: Allow all reagents and specimens to come to room temperature. Mix all reagents gently before using. Calibrator and samples are to be used undiluted.

Automated Method (Example)

Chemistry Parameters for Automatic Analyzer:
CRP (3) with Calibrator D, Standard Sensitivity

INSTRUMENT	Hitachi 717
TEMPERATURE	37°C
TEST	(CR3D)
ASSAY CODE	(2 POINT) : (28) - (44)
SAMPLE VOLUME	(3) (3)
R1 VOLUME	(150) (100) (NO)
R2 VOLUME	(150) (100) (NO)
WAVELENGTH	(800) (570)
CALIB. METHOD	(NONLINEAR) (4) (6)
STD.(1) Conc.-POS.	(0.00) - (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)
SD LIMIT	(100)
DUPLICATE LIMIT	(32000)
SENSITIVITY LIMIT	(0)
ABS. LIMIT (SLOPE)	(32000) (INCREASE)
PROZONE LIMIT	(-32000) (LOWER)
EXPECTED VALUE	(-99999) (99999)
PANIC VALUE	(-99999) (99999)
INSTRUMENT FACTOR	(1.0)

Use isotonic saline as STD (1)

* 2-6 Input concentration of calibrators

Parameters for other automated analyzers are available.

CALIBRATION

It is recommended that a 6-point calibration curve be made with saline (0 mg/L calibrator) and either the **K-*ASSAY*®** CRP (3) Calibrator D (standard sensitivity protocol), **K-*ASSAY*®** CRP (3) Calibrator E (high sensitivity protocol), or **K-*ASSAY*®** CRP (3) Calibrator F (wide sensitivity protocol). Please be sure to use the proper instrument application for the calibrator you select. It is recommended that the user determine calibration frequency, as this will depend on the instrument and type/number of other assays being run. Initially, calibration should be performed each day.

QUALITY CONTROL

It is recommended that control serum with a known concentration of CRP be included in all assay runs.

CALCULATIONS

CRP levels are determined by the analyzer using the prepared calibration curve.

LIMITATIONS OF PROCEDURE

Before starting the assay, the user must choose which protocol they desire with the K-*ASSAY*® CRP (3) assay. Each protocol has a different linear range and requires a specific calibrator set. It is important to note that each calibrator set has unique application parameters for use with the K-*ASSAY*® CRP (3) reagent. If the wrong application or wrong calibrator set is used, erroneous values may be obtained.

Standard Protocol: The **K-*ASSAY*®** CRP (3) has a measurable range from 0.1 to 320 mg/L (0.01 to 32.00 mg/dL) using the **K-*ASSAY*®** CRP (3) Calibrator D and standard parameters.

High Sensitivity Protocol: The **K-*ASSAY*®** CRP (3) has a measurable range from 0.05 to 160 mg/L (0.005 to 16.00 mg/dL) using the **K-*ASSAY*®** CRP (3) Calibrator E and high sensitivity parameters.

Wide Range Protocol: The **K-*ASSAY*®** CRP (3) has a measurable range from 0.2 to 480 mg/L (0.02 to 48.00 mg/dL) using the **K-*ASSAY*®** CRP (3) Calibrator F and wide range parameters.

Reagents should not be used after the expiration date indicated on the kit label. Do not mix reagents with different lot numbers.

If the CRP concentration is greater than highest calibrator value, dilute one part sample with four parts isotonic saline and re-assay. Multiply results by 5 to compensate for the dilution.

PERFORMANCE

The following performance data was obtained using a Hitachi 917 analyzer and standard protocol.

Sensitivity

When saline is used as a sample, the range of absorbance change per minute is -0.0050 to 0.0050, while a standard CRP solution containing 10 mg/L is 0.0650 to 0.1000 after subtracting the saline blank.

Specificity

When serum containing a known level of CRP is measured, the assay value obtained is within ± 10%.

Precision

Samples tested were commercial human CRP control serum.

Precision Assay:
(Within Run with CRP (3) Calibrator D)

	Sample I	Sample II	Sample III
N	20	20	20
Mean	0.48 mg/L	2.42 mg/L	9.80 mg/L
SD	0.02	0.04	0.09
CV	4.3%	1.6%	0.87%

Precision Assay:
(Between Runs with CRP (3) Calibrator D)
CRP values were tested on 21 days.

	Sample I	Sample II	Sample III
N	21	21	21
Mean	0.5 mg/L	2.2 mg/L	9.8 mg/L
SD	0.03	0.07	0.12
CV	6.97%	3.35%	1.23%

Accuracy / Correlation

y = 1.012x + .0051
r = 0.999
x = Company A's latex hsCRP nephelometric assay
y = **K-*ASSAY*®** CRP (3)

Assay Range

Standard Protocol:	0.1 to 320 mg/L (0.01 - 32.00 mg/dL)
High Sensitivity Protocol:	0.05 to 160 mg/L (0.005 - 16.00 mg/dL)
Wide Sensitivity Protocol:	0.2 to 480 mg/L (0.02 - 48.00 mg/dL)

Lower Limit of Detection

Standard Sensitivity Protocol:	0.10 mg/L (0.01 mg/dL)
High Sensitivity Protocol:	0.05 mg/L (0.005 mg/dL)
Wide Sensitivity Protocol:	0.20 mg/L (0.02 mg/dL)

Functional Sensitivity (lowest detectible concentration with a CV% of less than 20%)

Standard Sensitivity Protocol:	0.10 mg/L (0.01 mg/dL)
High Sensitivity Protocol:	0.05 mg/L (0.005 mg/dL)
Wide Sensitivity Protocol:	0.05 mg/L (0.005 mg/dL)

INTERFERENCE

Bilirubin C:	No interference up to 30 mg/dL.
Bilirubin F:	No interference up to 30 mg/dL.
Hemoglobin:	No interference up to 500 mg/dL.
Lipid:	No interference up to 5% Intrafat.
Rheumatoid Factor:	No Interference up to 560 IU/mL.

Dust particles or other particulate matter in the reaction solution may result in extraneous light-scattering, which may affect the accuracy of this test.

EXPECTED VALUES

Expected value for CRP in healthy individuals is from 0.105 to 2.51 mg/L. This value was calculated using 612 healthy adults. It is recommended that each laboratory establish its own expected range.

REFERENCES

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LABELING SYMBOLS

LOT	Lot Number
RGT	Reagent
	Expiration or "Use By" Date
REF	Catalog Number
IVD	For *In Vitro* Diagnostic Use
2-8°C	Temperature Limitation. Store between 2 and 8 degrees C
	Potential Human Biohazard
	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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USA patents 6248597, 6828158 European patent 0898169