K-*ASSAY*®

CRP (L)

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

Cat. No. KAI-033

INTENDED USE

For the quantitative determination of C-reactive protein (CRP) in serum by immunoturbidimetric assay. Measurement of CRP aids in the evaluation of the amount of injury to body tissues. FOR *IN VITRO* DIAGNOSTIC USE.

INTRODUCTION AND SUMMARY

C-reactive protein 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 or liver disease such as hepatitis A, hepatitis B, or biliary cirrhosis, and after severe infections such as septic shock.

The **K-*ASSAY*®** CRP 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

Antibodies specific for human CRP combine with CRP in the patient's serum forming immune complexes. The immune complexes cause an increase in light scattering, which correlates with the concentration of CRP in the serum. The light scattering is measured by reading turbidity at 340 nm and 700 nm. Polyethylene glycol in the reagent accelerates and enhances the complex formation.

KIT COMPOSITION

Reagents (Liquid stable)

R-1: Buffer Reagent, pH 7.6 100 mM Tris(hydroxymethyl)aminomethane	3 x 80 mL
R-2: Antiserum Reagent, pH 7.6 10% Anti-human CRP, goat antiserum 100 mM Tris(hydroxymethyl)aminomethane	1 x 60 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 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.

This product contains a chemical known to the State of California to cause cancer. The R-1 reagent contains 0.38% of thiourea (CAS No. 62-56-6).

REAGENT PREPARATION

Reagents are ready to use and do not require reconstitution.

STORAGE AND HANDLING

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

REAGENT STABILITY

Opened reagents can be used for 1 month if stored at 2-8℃. Discard reagents if they become contaminated. Evidence of cloudiness or particulate material in solution is cause to discard.

SPECIMEN COLLECTION AND PREPARATION

Serum test samples must be collected in the manner routinely used for clinical laboratory tests. Freshly drawn serum is preferred, but samples may also be stored refrigerated (2-8℃) for one week. Serum samples stored for extended periods should be frozen.

For storage of samples for more than a few days, use of plastic tubes is recommended instead of glass.

AUTOMATED ANALYZER APPLICATION

Suitable for two-reagent automated analyzers that use a six-point calibration method. Measurements of absorbance are to be made with a spectrophotometer able to accurately read absorbance at 340 and 700 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

PROCEDURE

Materials Supplied

KAI-033

Reagent 1 (R-1) Buffer Reagent	3 x 80 mL
Reagent 2 (R-2) Antiserum Reagent	1 x 60 mL

Materials Required But Not Supplied

Calibrators: **K-*ASSAY*®** CRP Calibrator, Cat. No. KAI-012C, 6 calibrators; Approx. values: 0, 0.9, 3.3, 6.7, 13.4, 24.4 mg/dL (For actual values see Package Insert).

Two Reagent Clinical Chemistry Analyzer:
Capable of accurate absorbance readings at 340 / 700 nm
Capable of accurately dispensing the required volumes
Capable of maintaining 37℃

Isotonic saline

Pipettes: capable of accurately dispensing the required volumes

Test Tubes: plastic or glass (for short term storage only)

Assay Procedure

An example of automated application (Hitachi 717):

Sample	12 µL
↓	
• ← R-1 (Buffer Reagent)	280 µL
↓ 37℃, 5 min	
• ← R-2 (Antiserum Reagent)	70 µL
↓ 37℃, 5 min	
2-point endpoint, 340/700 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

INSTRUMENT	Hitachi 717
TEMPERATURE	37°C
TEST	(CRP)
ASSAY CODE	(2 POINT) : (24) - (50)
SAMPLE VOLUME	(12) ( )
R1 VOLUME	(280) ( ) (NO)
R2 VOLUME	(70) ( ) (NO)
WAVELENGTH	(700) (340)
CALIB. METHOD	(NONLINEAR) (1) (6)
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)
SD LIMIT	(999)
DUPLICATE LIMIT	(10000)
SENSITIVITY LIMIT	(0)
ABS. LIMIT (SLOPE)	(32000) (INCREASE)
PROZONE LIMIT	(-32000) (LOWER)
EXPECTED VALUE	(-99999) (99999)
PANIC VALUE	(-99999) (99999)
INSTRUMENT FACTOR	(1.00)

* 1-6 Input concentration of calibrators

Parameters for other automated analyzers are available.

CALIBRATION

This kit does not include a calibrator. It is recommended that a multi-point calibration curve be made using the **K-*ASSAY*®** CRP Calibrator. 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 commercially available control serum with a known concentration of CRP be included in all assay runs.

CALCULATIONS

CRP levels are determined using the prepared calibration curve.

LIMITATIONS OF PROCEDURE

The **K-*ASSAY*®** CRP has a measurable range between 0.1 and 25 mg/dL. 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 the 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

Precision

When a sample that has a CRP concentration of around 3.8 mg/dL is assayed 20 times (within-run), the absorbance C.V. is below 5%. Precision data was gathered on a Hitachi 704 auto-analyzer. Samples tested were normal human serum spiked with known concentration of human CRP.

Precision Assay:
(Within Run)

Sample I	Sample II	Sample III
N = 20	N = 20	N = 20
Mean = 0.646	Mean = 3.147	Mean = 10.354
SD = 0.0278	SD = 0.0399	SD = 0.1052
CV = 4.30%	CV = 1.27%	CV = 1.02%

Precision Assay:
(Between Runs)
CRP values were tested on 6 days over a 1 month period.

Sample I	Sample II	Sample III
N = 6	N = 6	N = 6
Mean = 1.97	Mean = 5.33	Mean = 11.08
SD = 0.10	SD = 0.10	SD = 0.2
CV = 5.25%	CV = 1.94%	CV = 0.68%

Accuracy / Correlation

y = 0.9737x + 0.0003
r = 0.99838
x = ITA (company A)
y = **K-*ASSAY*®** CRP Assay

Assay Range

0.1 - 25 mg/dL

INTERFERENCE

Bilirubin:	No interference up to 20 mg/dL.
Hemoglobin:	No interference up to 500 mg/dL.
Lipemia:	No interference up to 5%.

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 ≤ 0.4 mg/dL. This value was calculated using 134 healthy adults. It is recommended that each laboratory establish its own expected range.

REFERENCES

1. Osmond, A.P., *et al. Proc. Natl. Acad. Sci.* 74:739-743, 1977.
2. Pepys, M.B. *Lancet*. 1:653-657, 1981.
3. Schultz, D.R. and P.I. Arnold. *Semin. Arthritis Rheum.* 20(3): 129-147, 1990.
4. Killingsworth, L.M. and J. Savory. *J. Clin. Chem.* 19:403-407, 1973.
5. Lizana, J. and K. Helling. *Clin. Chem.* 20:1181, 1974.
6. Otsuji, S., *et al. Clin Chem.* 28:2121-2124, 1982.
7. Malkus, H., *et al. Clinica Chimica Acta*, 88:523-530, 1978.

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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