K-*ASSAY*®

hsCRP

For the Quantitative Determination of Human CRP in Serum or Plasma

Cat. No. KAI-160

INTENDED USE

The **K-*ASSAY*®** high sensitivity C-reactive protein (hsCRP) assay is for the in vitro quantitative determination of C-reactive protein (CRP) in human serum and plasma on automated clinical chemistry analyzers. Measurement of CRP is of use for the detection and evaluation of inflammatory disorders and associated diseases, infection and tissue injury. For *in vitro* diagnostic use only.

CLINICAL SIGNIFICANCE

CRP (C-reactive protein, MW=25106Da) is an acute phase protein whose concentration is seen to increase as a result of the inflammatory process, most notably in response to pneumococcal (bacterial) infectious, histolytic disease, and a variety of disease states. Originally discovered by Tillet et al. in 1930 in patient sera with acute infection, CRP has now come to be used as a marker or general diagnostic indicator of infections and inflammation, in addition to serving as a monitor of patient response to therapy and surgery. Furthermore, regular measurements of CRP in infants can be a useful aid in the early diagnosis of infectious disease.[7]

PRINCIPLE OF TEST

The **K-*ASSAY*®** hsCRP assay is based on a latex enhanced immunoturbidimetric assay. When an antigen-antibody reaction occurs between CRP in a sample and anti-CRP which has been sensitized to latex particles, agglutination results. This agglutination is detected as an absorbance change (570 nm), with the magnitude of the change being proportional to the quantity of CRP in the sample. The actual concentration is then determined by the interpolation from a calibration curve prepared from calibrators of known concentration.

KIT COMPOSITION

Reagents (Liquid Stable)

R-1: Buffer Reagent
100 mM Tris-buffer solution
0.09% Sodium Azide

R-2: Latex Suspension, pH 6.0
Suspension of latex particles (≤0.5%) coated with goat anti-human CRP
0.09% Sodium Azide

WARNINGS AND PRECAUTIONS

FOR *IN VITRO* DIAGNOSTIC USE. Rx only.

Store the reagents at 2-8℃. DO NOT FREEZE. DO NOT INGEST. Avoid contact with skin and eyes. Contains sodium azide, which may react with lead or copper plumbing to form explosive compounds. Flush drains with copious amounts of water when disposing of this reagent. Specimens containing human sourced materials should be handled as if potentially infectious, using safe laboratory procedures such as those outlined in Biosafety in Microbiological and Biomedical Laboratories (HHS Publication Number [CDC] 93-8395). Additional safety information concerning storage and handling of this product is provided within the Material Safety Data Sheet for this product. To obtain an MSDS, please contact our diagnostics technical support department.

REAGENT PREPARATION

1. The **K-*ASSAY*®** hsCRP reagents are provided ready-to-use.
2. Physiological saline is needed to dilute high CRP samples and is used as a zero calibrator.

REAGENT STABILITY AND STORAGE

The **K-*ASSAY*®** hsCRP assay reagents should be stored at 2-8℃. DO NOT FREEZE. The reagents are stable when stored as instructed until the expiration date on the label. Do not mix reagent components from different lots.

SPECIMEN COLLECTION AND PREPARATION

Serum or heparinized plasma or EDTA plasma samples can be used for the hsCRP assay. For serum, collect whole blood by venipuncture and allow clotting. For plasma, mix the sample by gentle inversion prior to centrifugation. Centrifuge and separate serum or plasma as soon as possible after collection.

Sample stability[6]: 11 days at room temperature (15-25℃); 2 months at 2-8℃; and 3 years at -20℃

It is recommended that frozen samples are thawed at room temperature; samples must be mixed well before analysis. Repeated freezing and thawing should be avoided.

PROCEDURE

Materials Supplied

KAI-160 hsCRP

Reagent 1 (R-1) Buffer Reagent	1 x 50 mL
Reagent 2 (R-2) Latex Suspension	1 x 10 mL

Materials Required But Not Supplied

Calibrators: **K-*ASSAY*®** hsCRP Calibrator, Cat. No. KAI-161C (4 calibrators containing known amounts of human CRP)

Saline, used for diluting serum samples and as a zero calibrator

Controls such as **K-*ASSAY*®** hsCRP Controls, Cat. No. K80C-4M

Two-Reagent Clinical Chemistry Analyzer:
Capable of accurate absorbance readings at around 570 nm
Capable of accurately dispensing the required volumes
Capable of maintaining 37℃

Assay Procedure

CRP should be measured according to the specific application parameters for each specific chemistry analyzer. Below is a general example of the assay test scheme and the specific application parameters for the Hitachi 917 analyzer.

Sample, 5.0 µL
↓
• ← R-1 (Buffer Reagent), 250 µL
↓ 37℃, 5 min.
• ← R-2 (Latex Suspension), 50 µL
↓ 37℃, 1 min.
• → A1 Read, 570nm
↓ 37℃, 4 min.
• → A2 Read, 570nm

Calculate CRP value with the read absorbance change from a calibration curve prepared with calibrators of known concentrations. Application sheets for use of the Diazyme hsCRP assay on other automated clinical chemistry analyzers are available upon request. Please contact our diagnostics technical support department.

Automated Method (Example)

Chemistry Parameters for Automatic Analyzer

INSTRUMENT	Roche / Hitachi 917
TEMPERATURE	37°C
TEST	(hsCRP)
ASSAY CODE	(2 POINT END)(10) (19)(31)(0)(0)
WAVELENGTH	(800) (570)
SAMPLE VOLUME	(5.0) (0.0) (0)
R-1 VOLUME (R1)	(250) (0)
R-2 VOLUME (R3)	(50) (0)
ABS. LIMIT (SLOPE)	(32000) (INCREASE)
PROZONE LIMIT	(0) (0) (LOWER)
CALIB. TYPE	(SPLINE)
POINT	(5)
SPAN POINT	(5)
SD LIMIT	(999)
DUPLICATE LIMIT	(200)
SENSITIVITY LIMIT	(0)
S1ABS RANGE	(-32000) (32000)
INSTRUMENT FACTOR	a=(1.0) b=(0.0)
UNIT	(mg/L)
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)

Use saline for STD.(1). * 2-5 Input concentration of calibrators

Parameters for other automated analyzers are available.

CALIBRATION

K-*ASSAY*® hsCRP calibrators (Cat. No. KAI-161C) are available separately as a set of 4 ready to use levels. For automated analyzers, use saline and the provided calibrator 1-4 for calibration. The calibration curve is stable for at least 14 days.

QUALITY CONTROL

We recommend that each laboratory use CRP controls to validate the performance of hsCRP reagents. A set of **K-*ASSAY*®** High-Sensitive CRP Controls (Cat. No. K80C-4M) is available separately. The range of acceptable control limits should be established by individual laboratories.

RESULTS

Results are printed out in mg/L. Note: Samples with values greater than 20.0 mg/L should be diluted with saline and rerun. Multiply results by the dilution factor.

LIMITATIONS OF PROCEDURE

A sample with a CRP level exceeding the linearity limit of 20 mg/L should be diluted with 0.9% saline and reassayed incorporating the dilution factor in the calculation of the value.

PERFORMANCE

Precision

The intra-precision of the **K-*ASSAY*®** hsCRP Assay was evaluated as follows: in the study, three serum controls containing CRP were tested in duplicates on a Hitachi 917 over 20 days with 2 runs per day.

	Sample				
	Level 1	Level 2	Level 3	Serum	Serum
N	80	80	80	80	20*
Mean (mg/L)	0.85	1.75	8.62	3.62	15.56
Within Run S.D.	0.03	0.03	0.06	0.05	0.19
Within Run C.V. %	4.0	1.7	0.7	1.4	1.2
Total S.D.	0.04	0.05	0.12	0.09	0.24
Total C.V. %	4.2	2.6	1.4	2.4	1.6

*Sample was tested on Hitachi 917 over 5 days with 2 runs per day

Accuracy / Correlation

Correlation studies were performed by testing 57 serum samples with CRP concentrations ranging from 0.2 to 18.9 mg/L in comparison with an existing commercial CRP assay method. The linear regression gives a correlation r^2 value of 0.990, slope of 1.01, and y intercept of 0.0196.

LOB, LOD, and LOQ

The LOB, LOD, LOQ of the **K-*ASSAY*®** hsCRP Assay was determined on the Hitachi 917 according to CLSI EP17-A. By testing a True Blank Sample (7.5% BSA) in 20 replicates daily for 3 days, LOB was determined to be 0.08 mg/L. By testing five low serum samples (100x diluted) in 4 replicates for 3 days, LOD was determined to be 0.13 mg/L. To determine LOQ, specimens with mean measured concentrations ranging from 0.118 to 0.978 mg/L were assayed. Based on the EP evaluator-8 fitted model, the LOQ (lowest concentration for which CV is less than a target of 20% with 95% of confidence interval) is 0.20 mg/L CRP.

Linearity

CRP linearity set was prepared by diluting a specimen containing 40.0 mg/L CRP with saline according to CLSI EP6-A. Assay linearity was tested on the Hitachi 917. Data analysis using EP Evaluator 8 showed that the **K-*ASSAY*®** hsCRP assay was linear through a measured range of 0.20 to 20.0 mg/L with an allowable systematic error of 4.5%.

INTERFERENCE

The following substances do not interfere with this assay at the levels tested (less than 10% basis):

Ascorbic Acid:	No interference up to 176 mg/dL
Bilirubin, Conjugated:	No interference up to 40 mg/dL
Bilirubin, Unconjugated:	No interference up to 40 mg/dL
Hemoglobin:	No interference up to 500 mg/dL
Rheumatoid Factor:	No interference up to 400 IU/mL
Triglycerides:	No interference up to 1,000 mg/dL

EXPECTED VALUES

The assay reference interval was determined using serum specimens from 103 apparently healthy adults with ages of 18-62 according to CLSI C28-A3 guideline. The serum specimens were tested in duplicate by the **K-*ASSAY*®** hsCRP method. EP Evaluator 8 Software was used to verify the reference interval. The results showed that < 5.0 mg/L CRP was obtained in 95% of the population tested. However, it is recommended that each laboratory establish a range of normal values for the population it serves.

REFERENCES

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2. Du Clos TW: Function of C-reactive protein, Ann Med 32: 274-278, 2000.
3. Tietz, N.W. (Ed.), Clinical Guide to Laboratory Tests, 4th Edition, Alan H. Wu, Saunders Elsevier (2006).
4. Maksimowicz-McKinnon K, Bhatt DL, and Calabrese LH: Recent advances in vascular inflammation: C-reactive protein and other inflammatory biomarkers, Curr. Opin. Rheumatol 16: 18-24, 2004.
5. Pearson TA, Mensah GA, Alexander RW, et al. Markers of inflammation and cardiovascular diseases; application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation 2003; 107: 499-511.
6. Use of anticoagulants in Diagnostic laboratory Investigations. WHO Publication WHO/DIL/LAB 99.1/Rev. 2 Jan. 2002.
7. Benitz, W.E., et al., Serial serum C-reactive protein levels in the diagnosis of neonatal infection. Pediatrics 1998;102:E41

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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