MEDICA

IVD CE

REF 10228-4 4 x 9 mL / 9 mL

C-REACTIVE PROTEIN (CRP) REAGENT
Each wedge contains usable volumes of 9 mL of R1 reagent and 9 mL of R2 reagent.

INTENDED USE
The Medica CRP reagent is intended for use in the quantitative in-vitro diagnostic determination of C-reactive protein (CRP) in human serum or plasma using the EasyRA® Clinical Chemistry Analyzer. Measurement of C-reactive protein aids in evaluation of the amount of injury to body tissue.
For *in vitro* diagnostic use only. For professional use only.

SUMMARY AND EXPLANATION[1,2, 3]
C-reactive protein is present in serum or plasma of normal individuals at levels between 0-5 mg/L. Elevated levels are associated with acute phase response and measurements may be useful for the detection of infection, tissue injury, inflammatory disorders and associated diseases.

Increases in CRP values are non-specific and should not be interpreted without a complete evaluation of the patient's clinical history. CRP levels within the normal range may be affected by a number of different factors and should always be compared to previous values.

PRINCIPLE OF THE PROCEDURE
CRP is a turbidimetric immunoassay using two reagents sequentially. The serum or plasma sample is first reacted to a buffer (R1) containing bovine serum albumin, and then with latex particles coated with antibody to CRP (R2). The formation of the antibody-antigen complex during the reaction results in an increase in turbidity, which is measured as the amount of light absorbed at 600nm. The increase in absorbance is proportional to the concentration of the CRP in serum or plasma sample. By constructing a standard curve from the absorbance of the standards, the CRP concentration of sample can be determined using a Spline curve fitting routine.

REAGENTS

CRP Reagent (R1):

Glycine Buffer Reagent,	170 mM
Sodium Chloride	100 mM
Sodium EDTA disodium salt dihydrate	50 mM
Bovine Serum Albumin	1 %
Sodium Azide	< 0.1%

CRP Reagent (R2):

Latex particles coated with C-reactive protein antibody to CRP	
Sodium Azide	< 0.1%

PRECAUTIONS
1. Good laboratory safety practices should be followed when handling any laboratory reagent. (CLSI, GP17-A2).
2. This material contains Sodium Azide as preservative. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with your eyes or if ingested, see immediate medical attention. Refer to the Safety Data Sheet for risk, hazard and safety information.
3. As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
4. Do not use washed cuvettes.

INSTRUCTIONS FOR REAGENT HANDLING, STORAGE AND STABILITY
The reagent is ready to use as supplied. Unopened reagent is stable until the expiration date listed on the label if stored at 2 – 8°C. Reagents should be mixed thoroughly and equilibrated to the EasyRA system temperature for approximately 30 minutes prior to use on the analyzer. Do not use the R1 reagent if it is turbid or cloudy.

SPECIMEN COLLECTION AND STORAGE/STABILITY[4]

The specimen should be a fresh serum or plasma sample and should be stored refrigerated (2 – 8°C) until analyzed. Specimens can be stored up to 1 week at 2 – 8°C. If specimens need to be stored longer than 1 week before testing, they may be frozen at < -20°C for up to 6 months. Samples may be frozen only once. Refer to CLSI H18-A for further instructions on specimen collection, handling and storage.

PROCEDURE

Materials Provided:
Medica CRP Reagent Wedge, REF 10228-4

Additional Materials Required
Medica EasyCal CRP Calibrator Kit: REF 10659
Medica EasyQC® CRP Quality Control, Level 1: REF 10797
Medica EasyQC CRP Quality Control, Level 2: REF 10798
Medica EasyQC CRP Quality Control, Level 3: REF 10799
Medica Precision Test Dye Wedge, REF 10764
Medica Cleaner Wedge – Chemistry &ISE, REF 10660 *or*
Medica Cleaner Wedge – Chemistry, REF 10661

Instructions for Use
The reagent is ready to use as supplied. Remove the caps and place the reagent in the Medica EasyRA Chemistry Analyzer reagent tray located in the reagent area. When used this way, the reagent is stable on-board in the refrigerated area of the EasyRA analyzer for the number of days programmed on the RFID chip on the reagent wedge (61 days maximum).

Note: Check the inside of the necks of the wedge for foam after removing the caps and placing the wedge on the analyzer. If there is foam, remove it with a swab or a disposable pipette before performing the test. Use separate swabs or disposable pipettes for R1 and R2.

Calibration
Medica EasyCal CRP Calibrator Kit, (REF 10659) is recommended for the calibration of this assay. The concentration of CRP in unknown samples is derived from a calibration curve using an appropriate mathematical model such as Spline. The calibration curve is obtained with 6 calibrators (from the multi-calibrator set) at different levels. The corresponding calibrators values are listed in the package insert of the EasyCal CRP calibrator kit. The multi-point calibration interval (61 days maximum) is programmed on the RFID chip on the reagent wedge. Recalibration is required whenever there is a change in lot number, or if a shift in quality control values occurs.

Quality Control
Use the EasyQC CRP QC material Level 1(REF 10797), Level 2(REF 10798) and Level 3(REF 10799).
It is recommended that three levels of human serum based controls be run with the assay daily whenever patient testing is performed and with each reagent lot change. Failure to obtain the proper range of values in the assay of control material may indicate reagent deterioration, instrument malfunction, or procedural errors. The run should be repeated, making sure that all mixing and handling instructions are strictly followed. The laboratory should also follow local, state and federal quality control guidelines when using quality control materials.

Results
After completion of the assay, the EasyRA Analyzer calculates the CRP concentration of each sample. The concentration is derived from a multi-point calibration curve using an appropriate mathematical model such as Spline.

Expected Values
Each laboratory should establish its own range of expected values using this kit because differences exist among instruments, laboratories and local populations. The reported value for CRP is 0.5-160 mg/L, and the CRP concentration of an average specimen from a healthy individual should be $\leq$ 5 mg/L.[5]

Reportable Range and Procedural Limitations (e.g., if sample is above assay range)
The EasyRA Analyzer flags any result above 160 mg/L CRP as “LH” (Linear High). Samples with values above 160 mg/L should be retested. If the “Re-run” icon is selected by the operator, the sample may be retested using one-third (1/3) the sample volume. The retest results are calculated to reflect the use of the smaller sample volume. This will extend the reportable range of the CRP test to 480 mg/L. Samples below 0.5 mg/L CRP will be flagged with “LL” (Linear Low) and should be reported as <0.5 mg/L.

Performance characteristics

Reportable Range
The reportable range is 0.5 – 160 mg/L. Extended range is 0.5 to 480 mg/L when one-third of the sample is used (1:2 dilution).

Inaccuracy / Correlation (CLSI, EP9-A2)
The following table lists the data obtained in a comparison of the Medica Reagent for CRP (Y) on the EasyRA Analyzer to the performance of a FDA cleared CRP reagent (X) on the Hitachi® 911 Analyzer* using serum samples. The data shown below represents single determinations of serum obtained on the EasyRA Analyzer vs. the average of two replicate values obtained on the Hitachi 911 Analyzer.

Serum:	Number of samples	67	Range of Samples	0.90 to 153.2 mg/L
	Slope	1.0001	y Intercept	-0.0175
	Correlation Coefficient	0.9988	Regression Equation	Y = 1.0001x -0.0175

***** *Hitachi is a registered trademark of Kabushiki Kaisha Hitachi Seisakusho DBA Hitach, Ltd. CORPORATION JAPAN 6-6, Marunouchi 1-chome, Chiyoda-ku Tokyo JAPAN 100-8220.*

The following table lists the data obtained in a comparison of matched serum (X) and lithium heparin plasma (Y) samples using the Medica Reagent for CRP on the EasyRA Analyzer. The data in the table below represent mean values from the EasyRA.

Plasma:	Number of samples	51	Range of Samples	0.51 to 151 mg/L
	Slope	1.0021	y Intercept	-0.0067
	Correlation Coefficient	0.999	Regression Equation	Y = 1.0021x -0.0067

Imprecision (CLSI, EP5-A2)
Duplicate measurements of each level of QC material were tested twice a day for 20 days. Both total and within-run imprecision were calculated from these data.

Within run imprecision:

QC Level (mg/L)	Within Run SD (mg/L)	Within Run CV (%)
2.7	0.06	2.33
22.8	0.34	1.50
135.0	1.82	1.35

Total Imprecision:

QC Level (mg/L)	Total Imprecision SD (mg/L)	Total Imprecision CV (%)
2.7	0.09	3.32
22.8	0.65	2.85
135.0	3.29	2.44

Linearity (CLSI, EP6-A)
Linear from 0.5 to 160 mg/L for CRP, based on the linear regression Y = 1.0086x + 0.0526

Limit of Blank (LOB):	0.04 mg/L	(CLSI, EP17-A)
Limit of Detection (LOD):	0.16 mg/L	(CLSI, EP17-A)
Limit of Quantitation (LOQ):	0.5 mg/L	(CLSI, EP17-A)

Interfering Substances (CLSI, EP7-A)
Less than 10% change in the value was classified as “no significant interference.”
The following substances were evaluated:
No significant interference was found in levels up to 1000 mg/dL of hemoglobin.
No significant interference was found in levels up to 30 mg/dL of bilirubin.
No significant interference was found in levels up to 27 mg/dL of conjugated bilirubin.
No significant interference was found in levels up to 1750 mg/dL of triglycerides (using Intralipid®*).
No significant interference was found in levels of up to 500 mg/dL of ascorbic acid.
No significant interference was found in levels of up to 1024 IU/ml of rheumatoid factor.
No Hook effect was observed up to a CRP concentration of 2800 mg/L.

Intralipid is a registered trademark of Pharmacia AB, Clayton, NC.

References

1. Kuller L.H., Tracy T.R., Shaten J., Mellahn E. (1996) Relation of C-reactive Protein and Cornonary Heart Disease in the MRFIT nest case-control study. American Journal of Epidemiology Vol 144 (6):537-547. .
2. Thomas S.G., Kienast J., Pyke S., Haverkate F., van de loo J. (1995) Hemostatic Factors and the risk of myocardial infarction or sudden death in patients with angina pectoris. New England Journal of Medicine 332: 635-641.
3. Kindmark CO. The concentration of C-reactive Protein in sera from healthy individuals. Scand J Clin Lab Invest 1972;229: 407-411.
4. Tietz, N.W. (Ed), Fundamentals of Clinical Chemistry, W.B. Saunders C., Toronto, 636-638, 937 (1970).
5. Tietz Clinical Guide to Laboratory Tests, 4th edition, Saunders Elsevier, St. Louis, MO 480-483 (2006).

EasyRA Assay Parameters (CRP)

Primary Wavelength (nm)	600
Secondary Wavelength(nm)	N/A
Reaction Type	Special Endpoint (Immunoassay)
Reaction Direction	Increase
Reagent Blank	No
Sample Blank	No
Reaction Time	10 min
Calibration interval (maximum)	61 days
Reagent on-board stability	61 days

Serum/Plasma

Sample volume (µl)	3.0
Diluent 1 volume (µl)	20
Diluent 2 volume (µl)	20
Reagent volume R1 (µl)	90
Reagent volume R2 (µl)	90
Decimal Places (default)	1
Units (default values)	mg/L
Units(SI)	mg/dL
Dilution Factor	1:2 (1/3 of sample used to extend measuring range)
Linearity	0.5-160 mg/L

Medica Corporation, 5 Oak Park Drive
Bedford, MA 01730-1413 USA

EC REP Emergo Europe, Prinsessegracht 20
2514 AP The Hague, The Netherlands