hsCRP/CRP

Porrima

C-reactive Protein (CLIA)

REF: PM037702 **100**

Intended use
The Porrima CRP assay is a Chemiluminescent Immunoassay (CLIA) for the quantitative determination of C-reactive protein (hsCRP/CRP) in **Human Serum**. For professional use only.

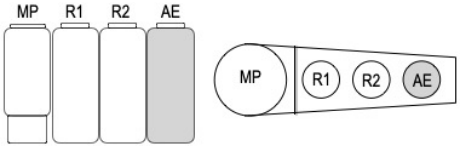
Summary
References[1-5]
C-reactive protein (CRP) is the classic acute phase protein in inflammatory reactions. It is synthesized by the liver and consists of five identical polypeptide chains that form a five-membered ring having a molecular weight of 105000 daltons. CRP is the most sensitive of the acute phase reactants and its concentration increases rapidly during inflammatory processes. Complexed CRP activates the classical complement pathway. The CRP response frequently precedes clinical symptoms, including fever. In normal healthy individuals CRP is a trace protein with a range up to 5 mg/L. After onset of an acute phase response the serum CRP concentration rises rapidly and extensively. The increase begins within 6 to 12 hours and the peak value is reached within 24 to 48 hours. Levels above 100 mg/L are associated with severe stimuli such as major trauma and severe infection (sepsis). CRP response may be less pronounced in patients suffering from liver disease.
Measuring changes in the concentration of CRP provides useful diagnostic information about how acute and how serious a disease is. It also allows judgements about the disease genesis.

Test principle
Two-step Sandwich principle. Total duration of assay: **25 minutes**
In the first incubation, sample, paramagnetic microparticles coated with monoclonal anti-CRP antibody and Buffer Solution are added into a reaction vessel. After incubation, microparticle is magnetically captured while other unbound substances are removed by washing.
In the second incubation, add monoclonal anti-CRP antibody-acridinium labeled conjugate to reaction mixture to form a sandwich complex, the complex becomes bound to the solid phase while other unbound Conjugate are removed by washing. Pre-Trigger and Trigger Solutions are then added to the reaction mixture, the resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of CRP in the sample and the RLUs detected by the Porrima system.

Materials provided

MP	1 x 5.0 mL, anti-CRP antibody (mouse, monoclonal) coated Microparticles in TRIS buffer with protein (bovine) stabilizers with preservative.
R1	1 x 5.0 mL, Buffer Solution
AE	1 x 10 mL, monoclonal anti-CRP antibody (mouse)-acridinium ester labeled conjugate in MES buffer with preservative.
C0	Calibrator, 1 vial, 1.0 mL, ready to use.
C1	Calibrator, 1 vial, 1.0 mL, ready to use.
C2	Calibrator, 1 vial, 1.0 mL, ready to use.

The position of each reagent component is shown in the figure below (front view on the left and top view on the right):



Materials required (but not provided)
- Porrima Chemiluminescent Immunoassay Analyzer
- CT2201 DiaSino ControlSet: 2 levels, L and H
- TR7701 Porrima TriggerPack: ①Pre-Trigger Solution, ②Trigger Solution
- WB7701 Porrima Wash Buffer (20X)
- RV7701 Porrima Reaction Vessels
- PW7701 Porrima Probe Wash Buffer
- SC7701 Porrima Sample Tubes (D13x75mm, D13x100mm or D16x100mm can be acceptable on Porrima system)
- Saline (0.85% to 0.90% NaCl) for specimens that require dilution

Precautions and warnings
- For in vitro diagnostic use only.
- Exercise the normal precautions required for handling all laboratory reagents.
- Disposal of all waste material should be in accordance with local guidelines.
- Do not use reagents beyond the labeled expiry date.
- Do not mix or use components from kits with different batch codes.
- All the specimen and reaction wastes should be considered potentially biohazard. The handling of specimens and reaction wastes should be in accordance with the local regulations and guidelines.
- Neutralized acids and other liquid waste should be decontaminated by adding a sufficient volume of sodium hypochlorite to obtain a final concentration of at least 1.0%. Exposure to 1.0% sodium hypochlorite for 30 minutes may be necessary to ensure effective decontamination.
- Some reagents contain 0.05% or 0.1% ProClin 300 which may cause sensitization by skin contact, which must therefore be avoided. Reagents and their containers must be disposed of safely. If swallowed, seek medical advice immediately and show this container or label.
- For information on hazardous substances included in the kit please refer to the Materials Safety Data Sheet (MSDS), which is available on request.
- Do not smoke, drink, eat or apply cosmetics in the work area.
- Do not pipette by mouth. Wear protective clothing, disposable gloves and eye/ face protection when handling samples and reagents. Wash hands after use.
- If any of the reagents comes into contact with the skin or eyes, wash the area extensively with water.

Reagent handling
The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.
All information required for correct operation is read in from the respective reagent barcodes.

Storage and stability
- Store at 2-8°C. Do not freeze.
- Store the Porrima reagent kit upright in order to ensure complete availability of the microparticles during automatic mixing prior to use.
- The Porrima CRP reagent kit can be stored onboard and used for a maximum of 28 days after opening at 2-8°C.

Specimen collection and preparation
- Human serum is recommended for this assay.
- Centrifuge the specimens after clot formation is complete. Transfer the supernatants into tubes for storage or test within two hours after centrifugation.
- Specimens should be tested as soon as possible after sample collection. If testing is not completed within 8 hours, specimens should be tightly capped and refrigerated at 2-8°C. If testing will be delayed for more than 72 hours, specimens should be frozen at -20°C or below.
- Avoid repeated freeze and thaw cycles.

Specimen Dilution Procedure (Manual Dilution)
Before assay, use saline (0.85% to 0.90% NaCl) to dilute the sample as 1:100, for example: add 20 µL of Sample (Serum) to 2.0 mL of Saline.

Assay procedure
- For optimal performance of this assay, operators should read the Porrima CLIA system operation manual carefully, to get sufficient information such as operation instructions, sample preservation and management, safety precaution, and maintenance. Prepare all required materials for the assay as well.
- Before loading the reagent kit on the machine for the first time, unopened reagent bottle should be inverted gently for at least 30 times to resuspend the microparticles that have settled during shipment or storage.
- Visually inspect the bottle to ensure the microparticles have been resuspended. If the microparticles remain adhered to the bottle, continue inverting until the microparticles have been completely resuspended.
- If the microparticles cannot be resuspended, **DO NOT USE**. Contact DiaSino Technical Support for help. Do not invert opened reagent bottle.
- This assay requires **20 µL of Sample (1:100 diluted Serum)** for a single test. This volume does not include the dead volume of the sample container. Additional volume is required when performing additional tests from the same sample. Operators should refer to the system operation manual and specific requirement of the assay to determine the minimum sample volume.

Calibration
Traceability: The Porrima CRP CLIA has been standardized against the IFCC (International Federation of Clinical Chemistry) standard CRM 470 (RPPHS).
The specific information of master calibration curve of CRP CLIA reagent kit is stored in the barcode attached in the reagent pack. It's used together with calibrators for the calibration of the specific reagent lot. When performing the calibration, scan the information of master calibration curve from the barcode into the system first, and then

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use the calibrators at two levels. Valid calibration curve is required before any CRP test. Recalibration is recommended every 28 days, or when a new reagent lot is used, or the quality controls are out of specified range. For detailed instruction of calibration, refer to the system operations manual.

Quality control
For quality control, use DiaSino ControlSet. In addition, other suitable control material can be used.
Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.
Quality control results should be within the acceptable ranges. If a control is out of its specified range, the associated test results are invalid and the samples must be retested. Recalibration may be required. Examine the assay system referring to the system operation manual. If the quality control results are still out of the specified range, please contact DiaSino Technical Support for help.

Calculation
The analyzer automatically calculates the analyte concentration of each sample in mg/L.

Limitations - interference
- The false positive results include cross-reactions with some components of serum from individual to antibodies, and non-specific adhesion of some components in human blood that have similar epitopes to capture and detector antibodies.
- In the case of false negative results, the most common factors are: non-responsiveness of antigen to the antibodies by that certain unknown components are masking its epitope, such that antigen cannot be seen by the antibodies; instability of CRP antigen, resulting in degradation with time and, or temperature, such that they become no longer recognizable by antibodies; and degraded other test components. The effectiveness of the test is highly dependent on storage of kits and sample specimens at optimal conditions.
- Other factors may interfere with Porrima CRP assay and may cause erroneous results. These include technical or procedural errors, as well as additional substances in blood specimens.
- For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Measuring range
0.5-300 mg/L (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 0.5 mg/L. Values above the measuring range are reported as > 300 mg/L.

Lower detection limit
0.5 mg/L
The detection limit represents the lowest analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1+2 SD, repeatability study, n = 21).

Expected values
0-5.0 mg/L
Expected values may vary with age, sex, diet and geographical location. Each laboratory should determine its own expected values as dictated by good laboratory practice. When CRP is used as an indicator for cardiovascular disease identification:

CRP level (mg/L)	Relative risk
< 1.0	Low
1.0-3.0	Average
> 3.0	High

Patients with higher hsCRP concentrations are more likely to develop myocardial infarction and severe peripheral vascular disease. It needs to be combined with the clinical diagnosis results of traditional cardiovascular disease indicators.
It is important to monitor the CRP concentration during the acute phase of the illness. Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data
Representative performance data are given below. Results obtained in individual laboratories may differ.

Precision
Precision was determined using DiaSino reagents, pooled human sera, and controls in a modified protocol (EP5-A) of the CLSI (Clinical and Laboratory Standards Institute): 2 times daily for 20 days (n = 40). The following results were obtained:

		Repeatability		Intermediate precision	
Sample	Mean mg/L	SD mg/L	CV %	SD mg/L	CV %
Human Serum 1	3.24	0.28	8.65	0.288	8.89
Human Serum 2	38.61	2.807	7.27	3.131	8.11
Human Serum 3	136.93	7.723	5.64	8.777	6.41
ControlSet 1	35.48	2.416	6.81	2.586	7.29
ControlSet 2	117.47	6.296	5.36	8.047	6.85

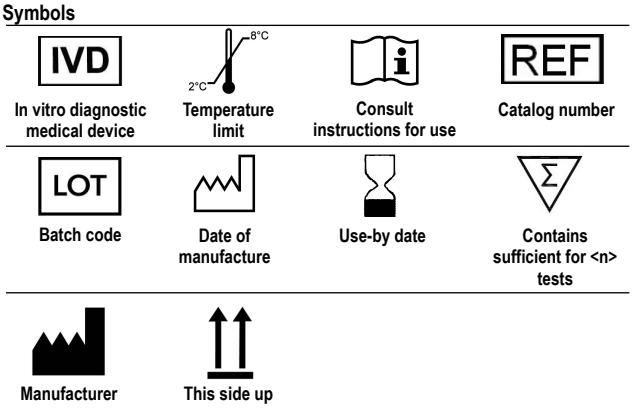
Method comparison
A comparison of the Porrima CRP assay (y) with the Beckman Coulter CRP assay (x) using 152 clinical samples gave the following correlations:
Linear regression
y = 1.015x - 0.098
r = 0.9758
The sample concentrations were between approx. 0 and 250 mg/L.

Functional sensitivity
0.55 mg/L
The functional sensitivity is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %.

References

1. Greiling, H., Gressner, A.M., eds. (1995). Lehrbuch der Klinischen Chemie und Pathobiochemie, 3rd ed. Stuttgart/New York: Schattauer Verlag, 234-236.
2. Thomas,L.(2008).LaborundDiagnose,7.Auflage,TH-BooksVerlagsgesellschaftmbH,Frankfurt/Main,1010-1021.
3. Burtis, C.A., Ashwood, E.R., eds. (2001). Tietz Fundamentals of Clinical Chemistry, 5th ed. Pa: WB Saunders Co, 332-333.
4. Thomas,L.,Messenger,M(1993).Pathobiochemie und Labordiagnostik der Entzündung.Labmed17,179-194.
5. Wasunna,A.,Whitelaw,A.,Gallimore,R.,etal.(1990).C-reactive protein and bacteria linfection in preterm infants. Eur JPediatr Mar149(6):424-427.

Symbols



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