MININEPH™ HUMAN C – REACTIVE PROTEIN KIT

For *in vitro* diagnostic use

Product Code: ZK044.L.R

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FDA (USA) Information (for MININEPH analyser only)

Analyte ID	C-reactive protein
Test System	The Binding Site MININEPH (MININEPH C-reactive protein)
Complexity Cat	Moderate

CE

1 INTENDED USE

This kit is designed for the *in vitro* measurement of human C-Reactive Protein (CRP) in serum using the MININEPH™ or MININEPHPLUS* as an aid in diagnosis and treatment of inflammatory conditions and bacterial infection.

**The MININEPHPLUS analyser is not available in the USA.*

2 SUMMARY AND EXPLANATION

C-Reactive protein (CRP), molecular weight 105kD, is one of a group of proteins called the pentaxins. It can readily bind to damaged cell membranes and microbial polysaccharides, and is involved in the agglutination and precipitation of invasive bacteria. It can also activate complement, resulting in inflammation, opsonisation and phagocytosis of cell debris and bacteria. CRP is synthesised in the liver and normal serum concentrations are very low (less than 10mg/L). Concentrations increase rapidly following inflammation and raised serum levels may be detected within six hours. It is probably the most useful and reliable indicator of the acute phase response, being preferable to the erythrocyte sedimentation rate (ESR). Moderately elevated serum levels (10-40mg/L) are associated with mild inflammation and viral infections. Higher concentrations (40 to 200mg/L) occur with acute phase inflammation and bacterial infections (refs 1,2,3).

3 PRINCIPLE OF THE ASSAY

The determination of soluble antigen concentration by nephelometric methods involves a reaction with the antibody bound to a latex particle to form insoluble complexes. When light is passed through the suspension formed, a portion of the light is scattered and detected by a photodiode. The amount of light scattered is directly proportional to the specific protein concentration in the test sample. Concentrations are automatically calculated by reference to a calibration curve stored within the instrument.

4 REAGENTS

4.1 MININEPH HUMAN CRP REAGENT
Consisting of monospecific antiserum coated onto polystyrene microparticles. Supplied in lyophilised form. It contains 0.099% sodium azide, 0.1% EACA, 0.01% Benzamidine and 0.05% ProClin™ as preservatives. Each vial should be reconstituted with 1.0mL of distilled water and allowed to stand for 30 minutes before use.
ProClin™ is a trademark of Rohm and Haas Corp. Philadelphia. PA

4.2 MININEPH CRP SWIPE CARD
This is encoded with details of the reaction curve specific to the respective lot of reagent. This card is reagent lot specific and must be used only with this lot of reagent. The curve on this card has been prepared using a secondary calibration material that is traceable to DA474.

4.3 MININEPH CRP BUFFER
For use with this lot of CRP reagent only. Contains 0.099% sodium azide as a preservative.

4.4 MININEPH HUMAN CRP HIGH AND LOW CONTROLS
These consist of pooled normal human serum and are supplied in lyophilised form. Reconstitute in 0.5mL distilled water and allow to stand for 15 minutes. They contain 0.099% sodium azide, 0.1% EACA and 0.01% benzamidine as preservatives. The acceptable ranges of CRP concentrations are stated on the Quality Control Certificate included in the kit. The lot number quoted on the Quality Control Certificate should be identical to the kit lot number.

5 CAUTION

All donors of human serum supplied in this kit have been serum tested and found negative for hepatitis B surface antigen (HBsAg) and antibodies to human immunodeficiency virus (HIV1 and HIV2) and hepatitis C virus. The assays used were either approved by the FDA (USA) or cleared for *in vitro* diagnostic use in the EU (Directive 98/79/EC, Annex II); however, these tests cannot guarantee the absence of infective agents. Proper handling and disposal methods should be established as for all potentially infective material, including (but not limited to) users wearing suitable protective equipment and clothing at all times. Only personnel fully trained in such methods should be permitted to perform these procedures.

WARNING: This product contains sodium azide and ProClin and must be handled with caution; suitable gloves and other protective clothing should be worn at all times when handling this product. Do not ingest or allow contact with the skin (particularly broken skin or open wounds) or mucous membranes. If contact does occur wash with a large volume of water and seek urgent medical advice. Explosive metal azides may be formed on prolonged contact of sodium azide with lead and copper plumbing; on disposal of reagent, flush with a large volume of water to prevent azide build up.

This product should only be used by suitably trained personnel for the purposes stated in the Intended Use. Strict adherence to these instructions is essential at all times. Results are likely to be invalid if parameters other than those stated in these instructions are used.

Reagents from different batch numbers of kits are **NOT** interchangeable. If large numbers of tests are performed care should be taken to ensure that all reagents are from the same batch.

6 STORAGE AND STABILITY

The unopened kits should be stored at 2-8°C and can be used until the expiry date given on the kit box label. DO NOT FREEZE. The buffer should be allowed to equilibrate to room temperature prior to use. Once opened the reagent and the controls should be stored at 2-8°C and the buffer at room temperature. Once reconstituted the reagent is stable for 2 weeks and the controls for 4 weeks when stored at 2-8°C. Opened buffer should be stored at room temperature and is stable for 3 months. The On-Board Buffer 1 should be stored at room temperature. Opened On-Board Buffer 1 is stable for 4 weeks when stored as recommended.

7 SPECIMEN COLLECTION AND PREPARATION

Use serum samples. Blood samples should be collected by venepuncture, allowed to clot naturally and the serum separated as soon as possible to prevent haemolysis. Sera may be stored at 2-8°C for up to two days, otherwise aliquot and freeze at –20°C or below; do not freeze and thaw sera more than once. Sample dilutions should be freshly prepared on the day of assay. Some types of sera are not suitable for MININEPH assay – see section 10.1.

8 METHODOLOGY

8.1 MATERIALS PROVIDED

8.1.1 2 x 1.0mL *MININEPH Human CRP Reagent*
8.1.2 1 x 25mL *MININEPH CRP Buffer*
8.1.3 1 x 0.5mL *MININEPH Human CRP High Control*
8.1.4 1 x 0.5mL *MININEPH Human CRP Low Control*
8.1.5 Magnetic swipe card containing lot specific calibration information
8.1.6 Quality Control Certificate
8.1.7 Instruction leaflet

8.2 MATERIALS REQUIRED BUT NOT PROVIDED (MININEPH)

8.2.1 MININEPH instrument (AD200)
8.2.2 MININEPH printer (AD210) (optional)
8.2.3 MININEPH reagent accessory pack (ZK500.R)
8.2.4 Electronic pipette (e.g. AD205)
8.2.5 A range of pipettes capable of dispensing 5-1000µL
8.2.6 Equipment for the collection and preparation of test samples
8.2.7 Distilled water

8.3 MATERIALS REQUIRED BUT NOT PROVIDED (MININEPHPLUS)

8.3.1 MININEPHPLUS instrument (AD500.C/D/E)
8.3.2 MININEPHPLUS PRINTER (AP1310DPK1T63) (optional)
8.3.3 Bar Code Reader (optional)
8.3.4 MININEPH reagent accessory pack (ZK500.R)
8.3.5 Pipette (5-1000µL)
8.3.6 Equipment for the collection and preparation of test samples
8.3.7 MININEPHPLUS On-Board Buffer 1 (SN107)
8.3.8 Pipette tips for use with the MININEPHPLUS – refer to MININEPHPLUS User Guide.

8.4 TEST PROCEDURE FOR MININEPH ANALYSER

8.4.1 Summary of reagent volumes added to the cuvette:

Reagent	Volume added
Sample (1/40 dilution)	20µL
MININEPH CRP Buffer	400µL
MININEPH Hu CRP Reagent	40µL

8.4.2 Switch the analyser and printer (if attached) on.
8.4.3 *Enter chemistry number*. Enter the chemistry number (CRP = 44) and press **enter**.
8.4.4 *Swipe chemistry card*. This message will only be displayed if this chemistry has never been used before or you wish to change reagent lot number. Pass the swipecard through the swipecard reader moving from the front of the instrument to the back. The magnetic strip should be at the bottom facing left.
8.4.5 *Check reagent lot number*. Press **enter.**
8.4.6 *CRP lot xxxx. OK? 1=Y 2=N*. Compare the details displayed with those on the reagent label. If the lot number displayed is identical to the first four digits of the lot number printed on the reagent vial, select Y (**press 1**) and continue to step 8.4.7. If the first four digits are different from those displayed select N (**press 2**) and return to step 8.4.4 to allow the details of the correct batch to be entered.
8.4.7 Prepare dilutions of controls and samples using the MININEPH Sample Diluent supplied in the MININEPH Reagent Accessory Pack (ZK500.R). The recommended sample dilution for CRP is 1/40 (e.g. pipette 20µL of sample into a sample dilution tube and add 780µL of sample diluent).
8.4.8 Prepare one MININEPH cuvette for each sample to be assayed. Using the forceps provided with the MININEPH place a stirring bar in each cuvette and then using a pipette add 20µL of diluted sample carefully to the bottom of each cuvette.
8.4.9 *Enter sample ID*. Enter an identity code (e.g. 1) for the first sample to be assayed then press **enter** to continue (refer to user manual for choice of identity codes).
8.4.10 *Sample dilution 1/40.* Accept the recommended dilution by pressing **enter,** or type in a new dilution factor if an alternative dilution is to be used.
8.4.11 *Place cuvette in chamber*. Place a cuvette containing a stirring bar and 20µL of diluted sample in the cuvette chamber. Press the cuvette down gently until it reaches the bottom of the chamber. The cuvette will be detected automatically.
8.4.12 *Add reagent.* Fill an electronic pipette with 400µL of MININEPH CRP Buffer and 40µL of MININEPH Hu CRP Reagent and dispense its contents into the cuvette. The MININEPH will detect the addition followed by movement of the stirring bar and the assay will begin. It is not necessary to press enter. After a 30 second blanking time the assay will take 60 seconds to complete, the result will then be displayed and printed automatically (if a printer is connected).
8.4.13 If the instrument indicates the result is higher than the intended measuring range, reassay the sample at a higher dilution of 1/440 (400µL MININEPH Sample Diluent + 40µL sample diluted 1/40). The sample dilution should be entered as 1/440 (see section 8.4.10).
8.4.14 If the instrument indicates the result is lower than the intended measuring range, reassay the sample at a lower dilution of 1/5 (160µL MININEPH Sample Diluent + 40µL sample). The sample dilution should be entered as 1/5 (see section 8.4.10).
8.4.15 On completion of the assay remove the cuvette and press **enter** to perform the next assay.
8.4.16 When all assays for the chosen chemistry have been completed press escape (**esc**) and select the chemistry number for the next set of assays.

8.5 TEST PROCEDURE FOR MININEPHPLUS ANALYSER

8.5.1 Summary of reagent volumes added to the cuvette:

Reagent	Volume added
Sample (1/40 dilution)	20µL
MININEPH CRP Buffer	400µL
MININEPH Hu CRP Reagent	40µL

8.5.2 Ensure that an empty waste pot is placed at the back of the MININEPHPLUS.
8.5.3 Attach a new pipette tip on the end of the MININEPHPLUS hand held pipette.
8.5.4 Check there is sufficient On-Board buffer 1 (SN107) in the drawer. There needs to be at least 10mL. *Refer to the* MININEPHPLUS *User Guide for instructions on replenishing the buffer.*
8.5.5 Switch on the analyser and printer (if attached).
8.5.6 *Enter chemistry number.* Enter the chemistry number (CRP=44) and press **enter**.
8.5.7 *Swipe chemistry card.* This message will only be displayed if this chemistry has never been used before or when changing reagent lot number. Pass the swipe card through the swipe card reader moving in a left to right direction across the front of the MININEPHPLUS with the magnetic strip facing upwards.
8.5.8 *Check reagent lot number. Press **enter**.*
8.5.9 *CRP lot xxxx. OK? 1=Y 2=N.* Compare the details displayed with those on the reagent label. If the lot number displayed is identical to that printed on the reagent vial, select YES (**press 1**) and continue to step 8.5.12. If the vial lot number is different from that displayed select NO (**press 2**) and return to step 8.5.8 to allow the details of the correct batch to be entered.
8.5.10 *Prime?* 1=Y 2=N. Prime the analyser to expel air bubbles in the plastic tube leading from the On-board buffer bottle to the hand-held pipette. This is done by pressing button **1** when prompted. Excess On-board buffer will be expelled into the waste pot. When priming has finished press **2.** Note that a prime will always be performed when starting a T1 assay that follows a T2 assay
8.5.11 *Pipette Y/N: Block Y/N*. There is a short period when the MININEPHPLUS stabilises its temperature.
8.5.12 Prepare dilutions of controls and samples using the MININEPH Sample Diluent supplied in the MININEPH Reagent Accessory Pack (ZK500.R). The recommended sample dilution for CRP is 1/40 (e.g. pipette 20µL of sample into a sample dilution tube and add 780µL of sample diluent).
8.5.13 Prepare one MININEPH cuvette for each sample to be assayed. Using the forceps provided with the MININEPHPLUS place a stirring bar in each cuvette and then using a pipette add 20µL of diluted sample carefully to the bottom of each cuvette.
8.5.14 *Enter sample ID.* Enter an identity code (e.g. 1) for the first sample to be assayed then press **enter** to continue (refer to user manual for choice of identity codes).
8.5.15 *Sample dilution 1/40.* Accept the recommended dilution by pressing **enter** or type in a new dilution factor if an alternative dilution is to be used.
8.5.16 *Place cuvette in chamber.* Place a cuvette containing a stirring bar and 20µL of diluted sample in the cuvette chamber. Press the cuvette down gently until it reaches the bottom of the chamber. The cuvette will be detected automatically.
8.5.17 *Supplementary buffer.* Using the MININEPHPLUS hand-held pipette, aspirate 400µL of MININEPH CRP buffer.
8.5.18 *Air Gap.* Using the MININEPHPLUS hand-held pipette, aspirate an air gap.
8.5.19 *Aspirate Reagent* Using the MININEPHPLUS hand-held pipette, aspirate 40µL of MININEPH Human CRP Reagent.
8.5.20 *Add Reagent.* Dispense the aspirated reagents into the cuvette. The stirring bar will rotate and the assay will begin. After a 30 second blanking time the assay will take 58 seconds to complete. The result will be displayed. Results will be automatically printed if a printer is connected.
8.5.21 If the instrument indicates the result is higher than the intended measuring range, reassay the sample at a higher dilution of 1/440 (400µL MININEPH Sample Diluent + 40µL sample diluted 1/40). The sample dilution should be entered as 1/440 (see section 8.5.15).
8.5.22 If the instrument indicates the result is lower than the standard measuring range, reassay the sample at a lower dilution of 1/5 (160µL MININEPH sample diluent + 40µL sample). The sample dilution should be entered as 1/5 (see section 8.5.15).
8.5.23 On completion of the assay remove the cuvette and press **enter** to perform the next assay.
8.5.24 When all assays for the chosen chemistry number have been completed press **esc** and select the chemistry number for the next set of assays.
8.5.25 Empty waste pot and discard the pipette tip from the hand held pipette.

8.6 QUALITY CONTROL

As with all good laboratory practice, users should run controls with every batch of samples.

9 INTERPRETATION OF RESULTS

9.1 Results are calculated by the instrument and displayed in mg/L. If a printer is attached the result is automatically printed out together with the patient identification code and the sample dilution. Further calculations are not necessary.
9.2 The approximate **measuring range** is 3.5-112 mg/L at a sample dilution of 1/40. The sensitivity limit is 0.44mg/L when using a 1/5 sample dilution. Sample concentrations higher than the stated range may result in antigen excess which can give misleading results; if this is suspected, samples should be reassayed at a 1/440 dilution (400µL MININEPH Sample Diluent + 40µL sample diluted 1/40).

10 LIMITATIONS OF PROCEDURE

10.1 SPECIFIC TEST LIMITATIONS

10.1.1 Nephelometric assays are not suitable for measurement of highly lipaemic or haemolysed samples or samples containing high levels of circulating immune complexes (CICs) due to the unpredictable degree of non-specific scatter these sample types may generate. Unexpected results should be confirmed using an alternative assay method.
10.1.2 The MININEPH assay has been optimised at an ambient temperature of 23°C. Significant variation from this temperature may affect the performance of the assay and we recommend that controls be run each day to monitor any possible effect.
10.1.3 Diagnosis cannot be made and treatment must not be initiated on the basis of CRP measurements alone. Clinical history and other laboratory findings must also be taken into account.

10.2 TROUBLE SHOOTING

Problem	Possible causes(s)	Suggested action(s)
Error message "Blank too high - reassay" displayed.	Very high analyte concentration. Lipaemic, turbid or haemolysed samples.	Reassay sample at a higher dilution. Try alternative assay method.
Controls out of range.	Product deterioration. Operator error.	Check expiry date. Repeat assay with the correct sample dilution.
Test sample giving unexpectedly low result.	Antigen excess.	Repeat assay at higher dilution. Check if the two results agree.

11 EXPECTED RESULTS

The following CRP results were obtained using normal adult donor sera and hospital patients on the MININEPH. Concentrations are in mg/L. We recommend local reference ranges are generated.

Sample type	Number of samples	Range
Normal Serum	72	All samples < 3.8
Clinical Serum	19	4.26 – 72.8

12 PERFORMANCE CHARACTERISTICS

12.1 PRECISION

12.1.1 Precision - MININEPH

CRP Precision Summary						
	Intra batch (n=30)		Day to day (n=10)		Inter instrument (n=5)	
	Mean mg/L	CV%	Mean mg/L	CV%	Mean mg/L	CV%
Serum 1	92.8	6.6	94.3	9.6	101.0	3.6
Serum 2	38.2	3.6	43.2	6.7	29.8	4.4
Serum 3	5.1	4.9	5.4	2.1	5.9	6.0

12.1.2 Precision –MININEPHPLUS

CRP Precision Summary						
	Intra batch (n=30*)		Day to day (n=10**)		Inter instrument (n=5***)	
	Mean mg/L	CV%	Mean mg/L	CV%	Mean mg/L	CV%
Serum 1	86.5	2.90	93.30	7.17	93.30	6.82
Serum 2	33.8	3.16	32.47	5.15	32.47	4.75
Serum 3	5.2	2.65	5.71	5.73	5.71	2.12

**These data represent the average coefficient of variation (CV) of three within-batch measurements repeated ten times at each concentration.*
*** Assays were performed at three different concentrations on ten separate occasions. The CV of the ten results at each concentration was calculated.*
**** Assays were performed at three different concentrations on each of five instruments. The CV of the five results at each concentration was calculated.*

12.2 COMPARISON STUDY

12.2.1 MININEPH

A correlation study was performed on 85 normal and clinical serum samples using this kit on a MININEPH and a commercially available reference method. The study demonstrated a good agreement yielding the following linear regression equation and correlation coefficient.

y = 0.932x - 0.908 mg/L (y = MININEPH CRP)
(x = Reference method)

correlation coefficient r = 0.995

12.2.2 MININEPHPLUS

42 normal and clinical adult sera were tested on the MININEPH and MININEPHPLUS. The study demonstrated a good agreement yielding the following Passing & Bablok equation and linear regression correlation coeffiecient:

y = 1.09x - 0.01mg/L (y = MININEPHPLUS CRP)
(x = MININEPH CRP)

correlation coefficient r = 0.996

13 REFERENCES

1. Protein Reference Unit Handbook of Clinical Immunochemistry (1999) 6th Edn. Ed A Milford Ward Publ. PRU Publications, Sheffield pp 109-111.
2. Rosen, M.A. (1990). C-Reactive Protein: a marker of infection, inflammation, tissue damage and malignancy. Diagnostic & Clin. Testing 28, 18-22.
3. Okamura, J.M. *et al* (1990). Potential clinical applications of C-reactive protein. J.Clin Lab-Analysis 4, 231-235.