MININEPH™ HUMAN RHEUMATOID FACTOR KIT

For *in vitro* diagnostic use

Product Code: ZK151.L.R

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FDA (USA) Information (for MININEPH analyser only)

Analyte Name	Rheumatoid Factor Immunological Test System
Complexity Cat	Moderate

CE

1 INTENDED USE

This kit is designed for the *in-vitro* measurement of human Rheumatoid Factor in serum, using the MININEPH or MININEPHPLUS*. Measurement of Rheumatoid Factor may aid in the diagnosis of rheumatoid arthritis.

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

2 SUMMARY AND EXPLANATION

Rheumatoid Factors (RF) are naturally occurring IgG, IgA or IgM class antibodies directed against the Fc portion of aggregated IgG molecules. The measurement of RF can be useful diagnostically, e.g. with rheumatoid arthritis the presence of RF indicates a poorer prognosis than in its absence. Elevated RF concentrations are frequently associated with a difficult clinical course and generalised disease in rheumatoid arthritis, however their presence is not restricted to rheumatic disease (refs. 1, 2).

3 PRINCIPLE OF THE ASSAY

The determination of soluble antibody concentration by nephelometric methods involves a reaction with the antigen 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 RF REAGENT
Consisting of aggregated (denatured) Human IgG coated onto polystyrene microparticles. Supplied in lyophilised form. It contains 0.099% sodium azide as a preservative. Each vial should be reconstituted with 1.0mL of distilled water and be allowed to stand for 30 minutes before use.

4.2 MININEPH RF 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 secondary calibration materials that have been evaluated against the WHO Reference Reagent Rheumatoid Arthritis Serum, Human NIBSC code: W1066.

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

4.4 MININEPH HUMAN RF HIGH AND LOW CONTROLS
These consist of pooled normal human serum and are supplied in stabilised liquid form. They contain 0.099% sodium azide as a preservative. The acceptable ranges of RF 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 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 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 when stored at 2-8°C. Opened buffer and controls are stable for 4 weeks when stored as recommended. 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 RF Reagent*
8.1.2 1 x 25mL *MININEPH RF Buffer*
8.1.3 1 x 0.5mL *MININEPH Human RF High Control*
8.1.4 1 x 0.5mL *MININEPH Human RF 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 RF Buffer	400µL
MININEPH Hu RF Reagent	40µL

8.4.2 Switch the analyser and printer (if attached) on.
8.4.3 *Enter chemistry number*. Enter the chemistry number (RF = 96) 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 *RF 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 RF 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 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 RF Buffer and 40µL of MININEPH Hu RF 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 100 seconds to complete, the result will then be displayed and printed automatically (if a printer is connected).
8.4.13 On completion of the assay remove the cuvette and press **enter** to perform the next assay.
8.4.14 If the instrument indicates the result is higher than the intended measuring range, reassay the sample at a higher dilution of 1/400 (180µL MININEPH Sample Diluent + 20µL sample diluted 1/40). The sample dilution should be entered as 1/400 (see section 8.4.10).
8.4.15 If the instrument indicates the result is lower than the intended measuring range, reassay the sample at a lower dilution of 1/11 (400µL MININEPH Sample Diluent + 40µL sample). The sample dilution should be entered as 1/11 (see section 8.4.10).
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 RF Buffer	400µL
MININEPH Hu RF 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 (RF=96) 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 swipecard through the swipe card reader 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 *RF lot xxxx. OK? 1=Y 2=N.* Compare the details displayed with those on the antiserum label. If the lot number displayed is identical to that printed on the antiserum 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 **s**upplied in the MININEPH Reagent Accessory Pack (ZK500.R). The recommended sample dilution for RF 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 RF 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 RF 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 98 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 1/400 (180µL MININEPH Sample Diluent + 20µL sample diluted 1/40). The sample dilution should be entered as 1/400 (see section 8.5.15).
8.5.22 If the instrument indicates the result is lower than the intended measuring range, reassay the sample at a lower dilution of 1/11 (400µL MININEPH Sample Diluent + 40µL sample). The sample dilution should be entered as 1/11 (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 IU/mL. 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 31–500 IU/mL at the recommended sample dilution 1/40. The assay sensitivity limit is 8.6 IU/mL when using a 1/11 sample dilution. Sample dilutions lower than 1/11 are not recommended.
9.3 Sample concentrations up to at least 3850 IU/mL will not result in antigen excess. Sample concentrations higher than this may give misleading results. If this is suspected, samples should be reassayed at a 1/400 dilution (180µL MININEPH Sample Diluent + 20µL 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 Diagnosis cannot be made and treatment must not be initiated on the basis of RF 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

120 normal adult donor sera were assayed for RF on the MININEPH and 97.5% of sera gave results below 19 IU/mL.
We recommend local reference ranges are generated.

12 PERFORMANCE CHARACTERISTICS

12.1 PRECISION

12.1.1 Precision - MININEPH

RF Precision Summary						
	Intra batch		Day to day		Inter instrument	
	Mean IU/mL	CV% (n=30*)	Mean IU/mL	CV% (n=30**)	Mean IU/mL	CV% (n=15***)
Serum 1	247	2.54	237	5.81	250	6.20
Serum 2	121	4.24	114	4.34	117	5.28
Serum 3	19.7	7.19	19.8	4.52	18.6	7.56

12.1.2 Precision – MININEPHPLUS

RF Precision Summary				
	Mean IU/mL	Intra batch CV% (n=30*)	Day to day CV% (n=30****)	Inter instrument CV% (n=25*****)
Serum 1	232	3.3	3.66	5.95
Serum 2	104	3.76	5.39	9.15
Serum 3	20.8	5.32	5.71	9.99

** These data represent the coefficient of variation (CV) of ten within-batch measurements at three analyte concentrations.*
*** Assays were performed at three different concentrations on 10 separate occasions. The CV of the 10 results at each concentration was calculated.*
****Assays were performed at three different concentrations on each of five instruments. The CV of the results at each concentration was calculated.*
*****Ten within-batch measurements were performed on three separate occasions and the overall CV for the thirty results at each concentration calculated.*
******Assays were performed five times at each concentration on five instruments. The overall CV of the 25 results at each concentration was calculated.*

12.2 LINEARITY

The linearity of this assay has been confirmed using a serially diluted serum sample. This gave a combined regression equation of $y = 0.973x + 10.90$, $R^2 = 0.999$, (y = measured RF concentration, x = theoretical concentration) over an observed range of 51-442 IU/mL.
At 1/11 sample dilution a regression equation of $y = 0.963x + 4.329$, $R^2 = 0.999$ was obtained over an observed range 20-122 IU/mL.
At 1/40 sample dilution a regression equation of $y = 0.953x + 12.29$, $R^2 = 0.999$ was obtained over an observed range of 51-229 IU/mL.

12.3 INTERFERENCE

Minimal assay interference (<10%) was demonstrated when pure preparations of IgA, IgM and IgG were added to a sample tested at a 1/11 dilution.

12.4 COMPARISON STUDY

12.4.1 MININEPH

A correlation study was performed on 42 clinical serum samples using this kit on a MININEPH and a commercially available reference method. The study demonstrated a good agreement giving the following linear regression equation and correlation coefficient.

y = 0.951x + 0.79 IU/mL (y = MININEPH RF)

(x = Reference method)

correlation coefficient r = 0.989

In addition 25 samples (weak Rose Waaler positive) giving values between 13.1-39.0 IU/mL were tested using this kit on a MININEPH instrument at 1/11 sample dilution and by a commercially available reference method.

The findings from both studies are summarised below

n= 67		Alternative RF kit +	Alternative RF kit -
MININEPH RF KIT	+	59	0
	-	5*	3
Relative sensitivity		92.2%	
Relative specificity		100%	
Relative agreement		92.5%	

* Different demographic populations were used to establish the normal ranges and this may account for the different interpretation of these five weak positive samples.

12.4.2 MININEPHPLUS

13 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 = 0.98x - 4.35 IU/mL (y = MININEPHPLUS RF)

(x = MININEPH RF)

correlation coefficient r = 0.997

13 REFERENCES

1. Waaler E (1940), On the Occurrence of a factor in human serum activating the specific agglutination of sheep blood corpuscles. Acta Pathol Microbiol Scand 17, 172-188.
2. Winchester R.J (1975) Characterization of IgG complexes in patients with rheumatoid arthritis. Ann NY Acad Sci 256, 73-81.