Freelite® Human Lambda Free Kit for use on the MININEPHPLUS™

For *in vitro* diagnostic use

Product Code: VK018

Product manufactured by:
The Binding Site Group Ltd., 8 Calthorpe Road, Edgbaston, Birmingham B15 1QT, UK.
www.bindingsite.co.uk
Telephone: +44 (0)121 456 9500
Fax: +44 (0)121 456 9749
E-mail: info@bindingsite.co.uk

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1 INTENDED USE

This kit is intended for the quantitation of lambda free light chains in serum on the MININEPHPLUS. Measurement of free light chains aids in the diagnosis and monitoring of multiple myeloma, lymphocytic neoplasms, Waldenström's macroglobulinemia, AL amyloidosis, light chain deposition disease and connective tissue diseases such as systemic lupus erythematosus in conjunction with other laboratory and clinical findings.

2 SUMMARY AND EXPLANATION

Immunoglobulin molecules consist of two identical heavy chains (α, δ, ε, γ or μ) which define the immunoglobulin class and two identical light chains (κ or λ). Each light chain is covalently linked to a heavy chain and the two heavy chains are linked covalently at the hinge region. In healthy individuals, the majority of light chain in serum exists in this form, bound to heavy chain. However, low levels of free light chain (FLC) are found in serum of normal individuals due to the over-production and secretion of FLC by the plasma cells. Whilst the molecular weight of both light chains is ≈22.5kD, in serum κ free light chain (κ-FLC) exists predominantly as a monomer and λ free light chain (λ-FLC) as a covalently linked dimer with a molecular weight of ≈45kD. This will lead to a differential glomerular filtration rate for κ-FLC and λ-FLC and may explain the observed ratio of κ-FLC to λ-FLC of 0.625 in serum compared to the ratio of bound κ to λ of 2.0.

Elevated serum levels of monoclonal FLC are associated with malignant plasma cell proliferation (eg. multiple myeloma), AL amyloidosis and light chain deposition disease. Raised serum levels of polyclonal FLC may be associated with autoimmune diseases such as systemic lupus erythematosus [1-13].

3 PRINCIPLE OF THE ASSAY

Evaluating the concentration of a soluble antigen by nephelometry involves the addition of the test sample to a solution containing the appropriate antibody in a reaction vessel or cuvette. A beam of light is passed through the cuvette and as the antigen-antibody reaction proceeds, the light passing through the cuvette is scattered increasingly as insoluble immune complexes are formed. Light scatter is monitored by measuring the light intensity at an angle away from incident light. The antibody in the cuvette is in excess so the amount of immune complex formed is proportional to the antigen concentration. Concentrations are automatically calculated by reference to a calibration curve stored upon the calibration card. The sensitivity of nephelometric assays can be increased by the use of particle enhancement [6]. This entails linking the antibody to a suitably sized particle that increases the relative light-scattering signal of the antigen-antibody reaction.

4 REAGENTS

4.1 Human lambda free reagent: Consisting of polyclonal monospecific antibody coated onto polystyrene microparticles. Supplied in lyophilised form, reconstitute with 0.75mL of distilled water and put on a roller mixer for 30 minutes before use. It contains 0.099% sodium azide, 0.05% ProClin™, 0.1% E-amino-n-caproic acid (EACA) and 0.01% benzamidine as preservatives.

4.2 Lambda free 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 the reagent lot stated.

4.3 Lambda free supplementary buffer: For use with the identical lot of Lambda Free reagent, swipe card and controls only. The supplementary buffer contains 0.099% sodium azide as a preservative.

4.4 Controls: These consist of pooled human sera that contain Lambda Free light chain. They are supplied in stabilised liquid form that contain 0.099% sodium azide, 0.1% EACA and 0.01% benzamidine as preservatives. The acceptable ranges of Human Lambda Free concentrations are stated on the batch specific Quality Control certificate (VIN018.QC) accompanying the kit.

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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 gloves, protective equipment and clothing at all times. Only personnel fully trained in such methods should be permitted to perform the procedures.

This product contains sodium azide and ProClin 300 and must be handled with caution. 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 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 the reagents are from the same kit lot.

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. Once reconstituted, the reagent is stable for 5 days when stored at 2-8°C. It is recommended that reagent bottles are used consecutively. The supplementary buffer should be allowed to equilibrate to room temperature prior to use. Opened supplementary buffer and controls are stable for 1 month when stored at 2-8°C.

7 SPECIMEN COLLECTION AND PREPARATION

Use fresh or deep frozen serum samples. Serum should be obtained by venepuncture, allowed to clot and the serum separated as soon as possible to prevent haemolysis. Samples may be stored at 2-8°C for up to 21 days, but for prolonged storage they should be kept frozen at -20°C or below. Repeated freeze/thaw cycles should be avoided. Microbially contaminated serum samples, samples containing particulate matter and lipaemic or haemolysed serum samples should not be used. Some types of sera are not suitable for MININEPHPLUS assays – see section 11.1.1

8 METHODOLOGY

Note: To enable full interpretation of results, free Kappa/Lambda ratios should be determined: samples must therefore be assayed using Binding Site's MININEPHPLUS **Freelite** Kappa Free Kit (VK016).

8.1 Materials provided

8.1.1 2 x 0.75mL *Human Lambda Free Reagent*
8.1.2 1 x 3.5mL *Lambda Free Supplementary Buffer*
8.1.3 1 x 0.5mL *Human Lambda Free High Control*
8.1.4 1 x 0.5mL *Human Lambda Free 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

8.2.1 MININEPHPLUS analyser (AD500.C/.D/.E)
8.2.2 MININEPHPLUS printer (AP1310DPK1T63) (Optional)
8.2.3 MININEPH Reagent Accessory Pack (ZK500.R)
8.2.4 MININEPHPLUS On-Board Buffer 1 (SN107)
8.2.5 A range of pipettes capable of dispensing 5-1000μL
8.2.6 Pipette tips for use with the MININEPHPLUS – refer to MININEPHPLUS User Guide.
8.2.7 Equipment for the collection and preparation of test samples
8.2.8 A roller mixer
8.2.9 Distilled water

8.3 Test procedure

8.3.1 Summary of reagent volumes added to the cuvette:

Reagent	Volume added
Sample (1/20 dilution)	40μL
Lambda Free Supplementary Buffer	100μL
Human Lambda Free Reagent	40μL

8.3.2 Ensure there is sufficient On-Board Buffer 1 to perform the tests. Refer to the MININEPHPLUS User Guide for instructions on replenishing the buffer.
8.3.3 Check that the waste pot is under the hand-held pipette stand at the back of the MININEPHPLUS and is empty.
8.3.4 Attach a pipette tip to the hand-held pipette and place back into the pipette holder.
8.3.5 Switch the analyser on.
8.3.6 *Enter chemistry number*. Enter the chemistry number (LAM = 18) and press **enter**.
8.3.7 *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 swipe card through the swipe card reader moving in a left to right direction across the front of the analyser. The magnetic stripe should be facing upwards.
8.3.8 *Check reagent lot number*. Press **enter.**
8.3.9 *LAM lot xxxx. OK? 1=Y 2=N.* Compare the details displayed with those on the reagent label and swipe card. If the lot number displayed is identical to the four digits of the lot number printed on the reagent vial and swipe card, select Y (press **1**) and continue to step 8.3.10. If the lot number is different from those displayed select N (press **2**) and return to step 8.3.7 to allow the details of the correct lot to be entered.
8.3.10 *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.**
8.3.11 *Block N Pipette N.* Allow analyser to warm to operating temperature. The correct operating temperature is coded in the calibration card.
8.3.12 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 Lambda is 1/20 (to prepare this dilution pipette 40μL of sample into a sample dilution tube and add 760μL of sample diluent).
8.3.13 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 40μL of diluted sample carefully to the bottom of each cuvette.
8.3.14 *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 Guide for choice of identity codes).
8.3.15 *Sample dilution 1/20.* Accept the recommended dilution by pressing **enter** or type in a new dilution factor if an alternative dilution is to be used.
8.3.16 *Place cuvette in chamber*. Place a cuvette containing a stirring bar and 40μL 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.
8.3.17 *Temperature Stabilising.* There is a waiting period of 90 seconds whilst the sample in the cuvette is warmed inside the analyser.
8.3.18 *Add reagent.* Using the MININEPHPLUS hand-held pipette, aspirate the Human Lambda Free Reagent
8.3.19 *Air Gap*. Using the MININEPHPLUS hand-held pipette, aspirate an air gap.
8.3.20 *Supplementary.* Using the MININEPHPLUS hand-held pipette, aspirate the Lambda Free Supplementary Buffer.
8.3.21 *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 150 seconds to complete. The result will be displayed. Results will be automatically printed if a printer is connected.
8.3.22 If the analyser indicates the result is higher than the intended measuring range (displayed as > or mg/L XS), re-assay the sample at a higher dilution of 1/200 (900μL MININEPH Sample Diluent + 100μL sample diluted 1/20). The sample dilution should be entered as 1/200 (see section 10.2).
8.3.23 On completion of the assay remove the cuvette and press **enter** to perform the next assay.

8.3.24 When all assays for the chosen chemistry have been completed press escape (**esc**) and select the chemistry number for the next set of assays.
8.3.25 Empty waste pot and discard the pipette tip from the hand held pipette.

8.4 Measuring range

All samples must be assayed first at the standard 1/20 assay dilution, giving an approximate measuring range of 4.91-98.3mg/L. For samples measuring over the upper limit of the curve with an analyser dilution of 1/20, the following dilution series should be used to minimise reagent usage.

Overall Dilution	Sample (µL)	Saline diluent (µL)	Lambda Approx measuring range (mg/L)
1/20 sample dilution	40µL of neat sample	760µL	4.91-98.30
1/200 sample dilution	100µL of 1/20 dilution	900µL	49.1-983.0
1/2000 sample dilution	100µL of 1/200 dilution	900µL	491-9830
1/20000 sample dilution	100µL of 1/2000 dilution	900µL	4910-98300

8.5 Antigen excess

All nephelometric assays can be susceptible to antigen excess with high concentration samples, leading to falsely low results. With **Freelite,** the amino acid composition of the free light chain produced by an individual B cell clone will influence the level at which a sample may show antigen excess. The MININEPHPLUS reaction kinetics can be used to assess the risk of antigen excess, which compares the results with limits set through testing of an extensive myeloma library. Any result with atypical reaction kinetics will give a "mg/L XS" flag and should be retested at a higher sample dilution to remove the antigen excess (see Section 8.4)

Important Note: A very small percentage of samples in antigen excess have normal reaction kinetics so will not prompt the "mg/L XS" flag. It is recommended that the following statement accompany all free light chain results.

"*Undetected antigen excess is a rare event but cannot be excluded. If these free light chain results do not agree with other clinical or laboratory findings, or if the sample is from a patient that has previously demonstrated antigen excess, the result must be checked by retesting at a higher dilution."*

9 QUALITY CONTROL

The controls provided should be included in all assay runs. The acceptable lambda free concentration ranges are stated on the accompanying Quality Control certificate (VIN018.DS). Results obtained during the run should only be accepted if the control results obtained are within the ranges stated.

Should a control measurement be out of range when assayed with a stored curve the control values should be re-measured. If repeat results are still out of range, the analyser should be checked before repeating the assay. If problems persist, refer to supplier.

10 INTERPRETATION OF RESULTS

10.1 Results are calculated by the analyser 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.
10.2 When diluted, the sample stability is not guaranteed. At 1/20 sample dilution; samples are stable for 2 hours at 21°C. Higher dilutions should be assayed immediately after preparation.

11 LIMITATIONS OF PROCEDURE

11.1 Specific test limitations

11.1.1 Nephelometric assays are not suitable for measurement of highly lipaemic or haemolysed samples or samples containing high levels of circulating immune complexes (CIC) due to the unpredictable degree of non-specific scatter these sample types may generate. Unexpected results should be confirmed using an alternative assay method.
11.1.2 Diagnosis cannot be made and treatment must not be given on the basis of free light chain measurements alone. Clinical history and other laboratory findings must be taken into account.
11.1.3 **Antigen excess**: See Section 8.5
11.1.4 Each monoclonal FLC contains unique amino acid combinations. It is therefore theoretically possible for certain monoclonal proteins to be undetectable by immunoassay leading to lower than expected measurements. In practice this occurs extremely rarely with the **Freelite** assay. Suspected samples should first be tested for antigen excess (see section 11.1.3 above) then further investigation by other laboratory methods (immunofixation and serum protein electrophoresis).
11.1.5 The nature of monoclonal proteins can cause a non-linear response in immunoassays, potentially leading to inconsistent results; this can be prevented by always diluting the samples in the sequence 1/20, 1/200, 1/2000, 1/20000. Omitting a dilution step should be avoided.
11.1.6 Due to the highly variable nature of monoclonal proteins, different reagent batches may react differently to the epitopes in some patient samples. In these instances, sample results may vary when tested using multiple batches Care should be taken when monitoring patients across multiple reagent lots. We recommend, wherever possible, that previous and current samples are tested on new reagent lots and the results compared.

11.2 Trouble shooting

Problem	Possible causes(s)	Suggested action(s)
Error message "Blank too high – reassay" displayed.	Very high analyte concentration.	Reassay sample at a higher dilution.
	Lipaemic, turbid or haemolysed samples.	Try alternative assay method.
Controls out of range.	Product deterioration.	Check expiry date.
	Operator error.	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.

12 EXPECTED RESULTS

The ranges provided below have been obtained from a limited number of samples and are intended for guidance purposes only. Wherever possible it is strongly recommended that local ranges are generated.

12.1 Adult serum ranges

282 normal subjects aged from 20 to 90 years were assayed using the Binding Site **Freelite** assays for the BN™II* [11]. The results are shown in the table below.

Normal adult serum	Mean conc.	Median conc.	95 Percentile range
Free kappa	8.36 (mg/L)	7.30 (mg/L)	3.30 - 19.40 (mg/L)
Free lambda	13.43 (mg/L)	12.40 (mg/L)	5.71 - 26.30 (mg/L)
	Mean	**Median**	**Total range**
Kappa/Lambda ratio	0.63	0.60	0.26 - 1.65

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13 PERFORMANCE CHARACTERISTICS

13.1 Precision

Lambda precision summary				
	Conc. (mg/L)	Intra-batch CV% (n=20*)	Day to day CV% (n=10**)	Inter-instrument CV% (n=5***)
Serum 1	80.54	5.1	6.7	6.1
Serum 2	29.42	3.9	3.8	5.1
Serum 3	7.62	3.2	7.4	6.5

*Intra-batch data: this data represents the coefficient of variation (CV) of twenty within-run measurements at three analyte concentrations.
**Day-to-day data: the measurements were performed on ten separate occasions and the overall CV of the results at each concentration was calculated.
***Inter-instrument data: assays were performed at three different concentrations, in duplicate on each of five instruments. The CV of the results at each concentration was calculated.

13.2 Linearity

The linearity of this assay was confirmed using a serially diluted polyclonal serum sample, which gave a regression plot of $y = 0.91x + 1.07$ *(mg/L),* $r^2 = 0.984$ (y = measured free lambda concentration, x = theoretical concentration).

13.3 Interference

Minimal assay interference by 200mg/L bilirubin (1.95%), 5.0g/L haemoglobin (4.90%) and (15000 formazine turbidity units) chyle (-7.41%) was demonstrated using a 57.1mg/L free lambda control serum.

13.4 Limit of Blank and Limit of Detection

The limit of quantitation and detection for this assay is defined as the lowest point of the calibration curve i.e. 4.91mg/L based upon a 1/20 sample dilution.

13.5 Comparison

95 normal adult sera and 30 clinical adult sera (from known/suspected multiple myeloma and systemic lupus erythematosus patients) were tested on the **Freelite** MININEPHPLUS and **Freelite** BNII assays. Results were as follows:

	Normal and clinical sera
Range (mg/L)	7.90 – 8680
Passing Bablok regression (mg/L)	1.03x – 1.36
linear regression R^2	0.9798

14 REFERENCES

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