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Freelite® Human Lambda Free kit for use on the SPAPLUS®

For *in vitro* diagnostic use

Product Code: LK018.S

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FDA (USA) Information
Analyte Name: Lambda Light Chains
Complexity Cat: Moderate

CE

Warning: The result of lambda free light chains in a given specimen determined with assays with different manufacturers can vary due to differences in assay methods and reagent specificity. The results reported by the laboratory to the physician must include the identity of the lambda free light chain assay used. Values obtained with different assay methods cannot be used interchangeably. If, in the course of serially monitoring a patient, the assay method used for determining lambda free light chain levels is changed, additional sequential testing should be carried out. Prior to changing assays, the laboratory **MUST** confirm baseline values for patients being serially monitored.

1 INTENDED USE

This kit is intended for the quantitation of lambda free light chains in serum and urine on Binding Site SPAPLUS. Measurement of free light chains in serum aids in the diagnosis and monitoring of multiple myeloma, lymphocytic neoplasms, Waldenström's macroglobulinaemia, AL amyloidosis, light chain deposition disease and connective tissue diseases such as systemic lupus erythematosus (SLE) in conjunction with other laboratory and clinical findings. Measurement of free light chains in urine aids in the diagnosis and monitoring of multiple myeloma, lymphocytic neoplasms, Waldenström's macroglobulinaemia, AL amyloidosis and light chain deposition disease 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 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.
FLC levels in urine are low. In a healthy kidney the tubular cells selectively reabsorb all FLC so their presence in urine is probably due to secretion into the urinary tract.
Elevated serum levels of monoclonal FLC are associated with malignant plasma cell proliferation (e.g. multiple myeloma), AL amyloidosis and light chain deposition disease. Raised serum levels of polyclonal FLC may be associated with autoimmune diseases such as SLE. The appearance of higher levels of FLC in urine may be indicative of kidney disease or malignant lymphoproliferative disease such as multiple myeloma. The monoclonal urinary FLC associated with lymphoid malignancy is called a Bence Jones protein [1-13].

3 PRINCIPLE

Evaluating the concentration of a soluble antigen by turbidimetry 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 decrease in intensity of the incident beam of light. The antibody in the cuvette is in excess so the amount of immune complex formed is proportional to the antigen concentration. A series of calibrators of known antigen concentration are assayed initially to produce a calibration curve of measured light scatter versus antigen concentration. Samples of unknown antigen concentration can then be assayed and the results read from the calibration curve.

The sensitivity of turbidimetric 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 **Latex reagent:** Consisting of polyclonal monospecific sheep antibody coated onto polystyrene latex. Preservative: 0.05% ProClin™, 0.1% E-amino-n-caproic acid (EACA) and 0.01% benzamidine.
4.2 **Calibrator and controls:** These consist of human sera that contain lambda free light chain. They are supplied in a stabilised liquid form and contain 0.099% sodium azide, 0.1% EACA and 0.01% benzamidine as preservatives.
4.3 **Supplementary reagent:** Containing 0.099% sodium azide as a preservative.

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 cleared 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 these 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 batch.

6 STORAGE AND STABILITY

The unopened kit should be stored at 2-8°C and can be used until the expiry date shown on the kit box label. DO NOT FREEZE. The latex reagent, calibrator, and control may be stored for up to three months after opening providing that they are capped to avoid evaporation and kept at 2-8°C in a refrigerator.
The *Human Lambda Free SPAPLUS Reagent* and *Lambda Free SPAPLUS Supplementary Reagent* may be stored, uncapped, on the analyser for up to 40 days, provided that the main power switch (located at the rear of the left hand panel) is left switched on.

7 SPECIMEN COLLECTION AND PREPARATION

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.[14] Repeated freeze/thaw cycles should be avoided. Microbially contaminated samples, samples containing particulate matter and lipaemic or haemolysed samples should not be used.

It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific sample stability criteria for its laboratory.[15]

8 METHODOLOGY

Note: to enable full interpretation of results, free kappa/lambda ratios should be determined; samples must therefore also be assayed using Binding Site's **Freelite** Kappa Free kit (LK016.S).

8.1 Materials provided

8.1.1 1 x 100 tests *Human Lambda Free SPAPLUS Reagent*
8.1.2 1 x 100 tests *Lambda Free SPAPLUS Supplementary Reagent*
8.1.3 1 x *Human Lambda Free SPAPLUS Calibrator Set* (6 x 1.0mL)
8.1.4 1 x 1.5mL *Human Lambda Free SPAPLUS Control*
8.1.5 1 x 1.5mL *Human Lambda Free SPAPLUS High Control*

8.2 Materials required but not provided

8.2.1 Equipment for collection and preparation of test samples e.g. sample tubes, centrifuge etc.
8.2.2 A fully operational and equipped SPAPLUS analyser.
8.2.3 Current analyser operating instructions: SPAPLUS Reference Guide, Insert Code FIN012.
8.2.4 Sample Diluent, 99: Dil 1 Pack Code: SN080.S
8.2.5 SPAPLUS Weekly Wash Protocol and Bottles: IK050.S

8.3 Reagent preparation

Before loading, gently mix by inversion ensuring no foam or bubbles are generated or remain on the surface as these may interfere with reagent aspiration.

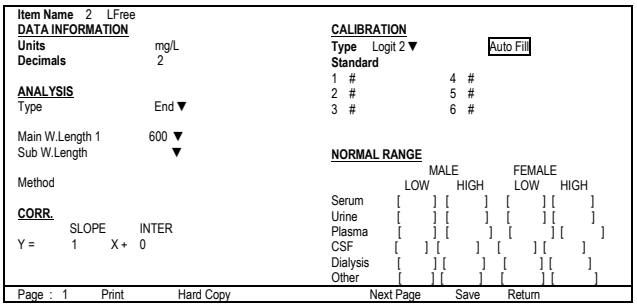
8.4 Test procedure

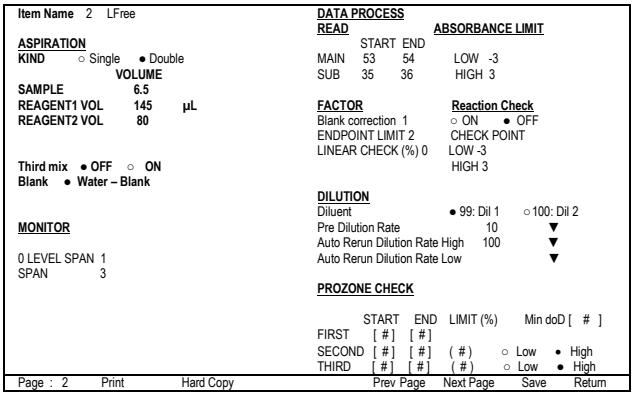
The user should be familiar with the operation of the SPAPLUS analyser before attempting to carry out the test procedures. The analyser should be prepared for use according to the manufacturer's instructions and the assay protocol entered as described below.

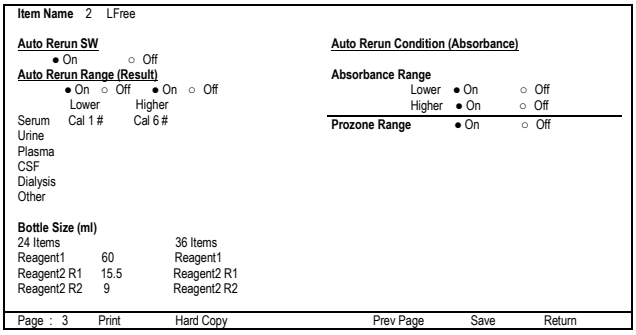
For full details of analyser operation refer to the SPAPLUS Reference Guide (FIN012) supplied with the analyser.

8.4.1 Test Parameters

Assay parameters are entered into Item Number 2.







The calibrator (Standard #), prozone check (#) and Min dOD values (#) are found in the Quality Control Certificate (SIN114.DS). Calibrator values on Page 1 should be entered in ascending order, i.e. the lowest value first. The prozone check and Min dOD values (#) should be entered on Page 2. The analyser will automatically calculate and enter the correct measuring ranges on Item pages 3 and 4 providing the Auto Fill button is pressed after typing the value for calibrator 6 on page 1. View Item parameter pages 3 and 4 to ensure correct value entry.

8.4.2 The SPAPLUS can be set up to automatically calculate the Free Kappa / Free Lambda ratio. Refer to the SPAPLUS Reference Guide (FIN012) for details of installing calculation parameters.

8.5 Special wash procedure for urine testing

Sample carryover can occur with free light chains in urine samples. To prevent this:

- **Freelite** urine tests must be batched together and run independently of other tests.
- Auto-dilutions must be turned off and repeats requested manually.
- Wash all cuvettes which have been used for urine dilutions or reactions using the weekly wash protocol. This needs to be done each time the cuvettes are used; this will allow a maximum of 30 tests in a single run (15 samples if kappa and lambda free light chains are both being tested).
- This wash protocol must be carried out after every run of urine samples and before any further testing is carried out.

This wash protocol comprises the SPAPLUS weekly wash using sodium hypochlorite.
Parameters for the hypochlorite wash step are provided with the SPAPLUS Weekly Wash Protocol and Bottles (IK050.S)

8.6 Measuring range

All samples must be assayed first at the standard 1/10 sample dilution, giving an approximate measuring range of 4.5-165mg/L. This enables a sensitivity of 0.45mg/L on neat serum samples. The upper limit of the measuring range using a sample dilution of 1/100 is 1650mg/L. For samples measuring over this limit, the following dilution series should be used:

Overall dilution	Analyser dilution	Manual pre-dilution	Approximate range (mg/L)
1/1	1/1	-	0.45 – 16.5
1/10	1/10	-	4.5 – 165
1/100	1/100	-	45 – 1650
1/1000	1/10	1/100*	450 – 16500
1/10000	1/100	1/100*	4500 - 165000

*Make a manual pre-dilution of 1/100 by taking 100µL of sample and add 900µL system sample diluent to achieve an initial 1/10 dilution. From this, take 100µL of this dilution and add 900µL system sample diluent to achieve a final 1/100 dilution. Present the 1/100 diluted sample for analysis. Multiply the result x 100.

8.7 Interpretation of results

The results of this assay should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings including previous **Freelite** results if available.

Due to the nature of monoclonal proteins, some samples may exhibit non linearity when assayed at different dilutions. In order to appropriately quantify such samples it is advised that the dilution protocol described in section 8.6 is followed and the first plausible result is reported.

All immunoassays have the potential for antigen excess. In order to identify samples that are in antigen excess the SPAPLUS has the facility to monitor reaction kinetics. Samples that demonstrate unusual reaction kinetics will generate a P flag. Samples that have generated a P flag must be repeated at a higher dilution as described in section 8.6 (note: the SPAPLUS will carry out auto-dilutions up to 1/100). If upon repeat the sample gives a result that is considered implausible, the samples should be repeated at the initial dilution, reviewed and reported.

Refer to the SPAPLUS Reference Guide (FIN012) supplied with the analyser for further details of flag interpretation.

Important Note: No automated check will identify all cases of antigen excess and a very small percentage of samples in antigen excess may not prompt the "P" 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 the 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. Results should always be interpreted in conjunction with other laboratory tests and clinical evidence; any anomalies should be discussed with the testing laboratory.

9 QUALITY CONTROL

At least two levels of appropriate control material should be tested a minimum of once a day. In addition, controls should be tested after calibration, with each new lot of reagent and after specific maintenance or troubleshooting steps described in the SPAPLUS Reference Guide
Quality control testing should be performed in accordance with local regulatory requirements and each laboratory's standard procedure.
Should a control measurement be out of range when assayed with a stored curve the assay must be recalibrated. If on recalibration the control values measured with the new curve are still out of range, the instrument and the assay parameters should be checked before repeating the assay. If problems persist, refer to your local technical support organisation.
The concentrations of the controls provided are stated on the accompanying QC certificate (SIN114.DS). Sample results obtained should only be accepted if the control results are within ±20% of the concentration(s) stated.

10 LIMITATIONS

10.1 Turbidimetric 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.
10.2 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.
10.3 This assay has not been established for use with the paediatric population.

11 EXPECTED VALUES

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.

11.1 Serum Reference Interval

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.

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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11.2 Urine Reference Interval

These ranges were obtained by measuring the free light chain concentrations of urines provided by 120 healthy adult donors on the SPAPLUS analyser. For both free kappa and free lambda measurements a number of samples ran below the measuring range of the assay.

Adult urine	Mean conc.	Median conc.	95 Percentile range
Free kappa	8.15 mg/L	4.93 mg/L	0.012 – 32.71 mg/L
Free lambda	0.93 mg/L	0.55 mg/L	< 0.45 – 4.99 mg/L

See section 12.2 for SPAPLUS serum correlation with the BNII assay and SPAPLUS urine correlation with the IMMAGE assay.

12 PERFORMANCE CHARACTERISTICS

12.1 Precision

Serum

A precision study was performed following CLSI guidelines *Evaluation of Precision Performance of Clinical Chemistry Approved Guideline* (EP5-A). The study was carried out over 21 working days, with two runs per day. One user assessed three different samples using three different reagent lots on three analysers. The following mean values were obtained for the samples tested:

Within-run precision

	Serum 1	Serum 2	Serum 3
Mean (mg/L)	10.35	35.09	142.10
CV%	3.4	2.4	2.0

Between-run precision

	Serum 1	Serum 2	Serum 3
Mean (mg/L)	10.35	35.09	142.10
CV%	2.2	0.0	2.4

Between-day precision

	Serum 1	Serum 2	Serum 3
Mean (mg/L)	10.35	35.09	142.10
CV%	7.2	4.4	6.8

Total precision

	Serum 1	Serum 2	Serum 3
Mean (mg/L)	10.35	35.09	142.10
CV%	8.2	5.0	7.4

Urine

A precision study was performed following CLSI guidelines *Evaluation of Precision Performance of Clinical Chemistry Approved Guideline* (EP5-A). The study was carried out over 21 working days, with two runs per day. One user assessed three different samples using one reagent lot on three analysers. The following results were obtained for the samples tested:

Within-run precision

	Urine1	Urine 2	Urine 3
Mean (mg/L)	7.54	48.95	149.55
CV%	3.7	1.8	2.8

Between-run precision

	Urine1	Urine 2	Urine 3
Mean (mg/L)	7.54	48.95	149.55
CV%	3.9	2.5	5.7

Between-day precision

	Urine1	Urine 2	Urine 3
Mean (mg/L)	7.54	48.95	149.55
CV%	5.7	5.6	6.9

Total precision

	Urine 1	Urine 2	Urine 3
Mean (mg/L)	7.54	48.95	149.55
CV%	7.8	6.4	9.4

12.2 Comparison

Serum: A correlation study was performed on 176 serum samples (120 normal, 21 from known SLE patients and 35 from known myeloma patients) using this kit on a SPAPLUS and on Binding Site **Freelite** BNII assay. The study demonstrated a good agreement giving the following Passing & Bablok comparison plot and linear regression correlation coefficient:

y = 1.003x + 0.0947mg/L (y = SPAPLUS)

(x = Reference method)

correlation coefficient r = 0.9977

Urine: A correlation study was performed on 124 urine samples (14 normal and 110 from clinical urine samples) using this kit on a SPAPLUS and on Binding Site **Freelite** IMMAGE assay. The study demonstrated a good agreement giving the following Passing & Bablok regression equation:

y = 1.03x + 0.14mg/L (y = SPAPLUS)

(x = Reference method)

12.3 Analytical sensitivity

Serum: Analytical sensitivity was determined by assaying ten replicates of two serum samples with concentrations equivalent to 140% and 200% of the lowest calibrator value. Two distinct sets of data were generated with CVs of 5.3% and 3.0% respectively.

Urine: An analytical sensitivity study was carried out according to CLSI guidelines EP17 Protocols for Determination of Limits of Detection and Limits Quantitation. The limit of quantitation is defined as the lowest amount of analyte that can be quantitatively determined; this has been calculated as 0.45 mg/L in urine.

12.4 Linearity

Serum: The linearity of this assay has been confirmed using a serially diluted serum sample, which gave a regression plot of *y = 0.9942x – 2.4692 (mg/L), r^2 = 0.9994* (y = measured free lambda concentration, x = theoretical concentration), over a measuring range of 3-976mg/L.

Urine: The linearity of this assay has been confirmed using a serially diluted urine sample, which gave a regression plot of *y = 0.9725x - 2.06 (mg/L), r^2 = 0.992* (y = measured free lambda concentration, x = theoretical concentration), over a measuring range of 2 - 405 mg/L

12.5 Interference

Serum: Minimal assay interference by 200mg/L bilirubin (-2.4%), 3g/L haemoglobin (-1.6%) and 0.3% Intralipid® (-3.0%) has been demonstrated using a 7mg/L free lambda control serum. Minimal interference (<9%) was also seen when adding purified IgG (10g/L), IgA (2g/L), IgM (1g/L) plus rheumatoid factor (320 IU/mL) to two serum samples containing high (150mg/L) and low (20mg/L) concentrations of free lambda.

Urine: Minimal assay interference by 200mg/L bilirubin (-5.4%), 240 mg/L haemoglobin (0.5%), 200 mg/L ascorbic acid (-1.7%) and 5 g/L albumin (8.9%) has been demonstrated using a 5mg/L free lambda urine.

12.6 Antigen excess

Polyclonal samples: The assay was tested to a level of 330mg/L with a polyclonal serum sample. No antigen excess was observed at this level.

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