1 CONTENTS

Page no.

Freelite® Human Lambda Free kit for use on the HITACHI 911/912/917/Modular P

For *in vitro* diagnostic use

Product Code: LK018.H

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

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 from 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 chains assay used. Values obtained with different assay methods cannot be used interchangeably. If, in the course of monitoring a patient, the assay method used for determining Lambda free light chains levels serially 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 the Roche Hitachi 911, Hitachi 912, Hitachi 917 and Modular P. Measurement of free light chain 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, β, 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 (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. 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 antibody coated onto polystyrene latex. Preservatives: 0.05% ProClin™, 0.1% E-amino-n-caproic acid (EACA) and 0.01% benzamidine.
4.2 **Standard 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 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 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 at 2-8°C for up to three months after opening providing precautions to prevent evaporation and contamination are taken.

7 SPECIMEN COLLECTION AND PREPARATION

Use fresh or deep frozen serum or urine samples. Serum should be obtained by venipuncture, 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 or urine samples, samples containing particulate matter and lipemic or haemolysed serum samples should not be used.

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.H).

8.1 Materials provided

8.1.1 2 x 6.5mL *Human Lambda Free Reagent (R2)*
8.1.2 1 x 25mL *Human Lambda Free Supplementary Reagent (R1)*
8.1.3 2 x 1.5mL *Human Lambda Free Standard*
8.1.4 1 x 1.0mL *Human Lambda Free Control Serum*
8.1.5 1 x 1.0mL *Human Lambda Free High Control Serum*

8.2 Materials required but not provided

8.2.1 Equipment for collection and preparation of test samples eg. sample tubes, centrifuge, etc.
8.2.2 Empty reagent bottles with barcode labels (available from Roche).
8.2.3 Special wash solution NaOHD, code 402 – 1822551 and 1551540 (available from Roche).
8.2.4 A fully operational and equipped Hitachi 911, 912, 917 or Modular P. A Hitachi 911 library disk with application codes 361-400.

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 Hitachi 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.

Complete installation instructions for both Hitachi 911, 912, 917 and Modular P analysers are available; please contact your local distributor for further information. Instructions denoted by an asterix indicate a user-determined parameter.

NB: The Lambda Free assay requires the installation of a saline calibrator for Calibrator 1 and Human Lambda Free Standard as Calibrators 2-6. Install both calibrators using any unassigned calibrator numbers between 900 and 999. If a saline calibrator has already been installed on the instrument this can be assigned to the Lambda Free assay.

8.4.1 Parameters (Hitachi 911)

Test [Lambda] [*****] Test Name [Lambda] Unit [mg/L]
Data Mode [On Board] Report Name [Lambda Free]

Control Interval [0] Instrument Factor(Y=aX+b) a[1.0]
b[0]

Expected Value (Serum) Expected Value (Urine)
Age (M) (F)
[0] [Y] [0] - [100] [0] - [100] [0] - [100]
[0] [Y] [0] - [100] [0] - [100]
[0] - [100] [0] - [100]

Technical Limit (Serum) (Urine)
[STD2 CONC] - [STD6 CONC] [0] - [100]

STD	Conc.	Pos.	Sample	Pre.	Dil.	Calib.	Lot No.	Qualitative	[No]
(1)	[0.00]	34	[5]	[0]	[0]	[034]	000001	(1) [0]	[ ]
(2)	[20% of Cal value]	**	[5]	[5]	[195]	[***]	000002	(2) [0]	[ ]
(3)	[50% of Cal value]	**	[10]	[5]	[150]	[***]	000000	(3) [0]	[ ]
(4)	[80% of Cal value]	**	[20]	[5]	[180]	[***]	000000	(4) [0]	[ ]
(5)	[160% of Cal value]	**	[20]	[5]	[80]	[***]	000000	(5) [0]	[ ]
(6)	[267% of Cal value]	**	[40]	[5]	[80]	[***]	000000	(6)	[ ]

Test [Lambda]
Assay Code [2 Point End] [10] [ ] Wavelength(2nd/Primary) [ ]/[600]
Assay Point [7] - [31] - [0] - [0] Diluent/Rgt. Stability [00301]/[0]

	(Serum)			(Urine)		
S. Vol. (Normal)	[20]	[5]	[140]	[1]	[0]	[0]
S. Vol. (Decrease)	[2]	[5]	[158]	[1]	[0]	[0]
S. Vol. (Increase)	[5]	[0]	[0]	[1]	[0]	[0]
ABS. Limit	[32000]	[32000]		[Increase]		
Prozone Limit	[32000]	[32000]		[Upper]		
Reagent R1	[150]	[0]	[*****]	[0]		
R2	[90]	[0]	[*****]	[0]		
R3	[0]	[0]	[*****]	[0]		
R4	[0]	[0]	[*****]	[0]		

Calibration Type [Logit-Log (4P)] [6] [6] [0] [ ]

Auto Time Out Blank [0] SD Limit [999]
Span [0] Duplicate Limit [32000]
2 Point [0] Sensitivity Limit [0]
Full [0] S1 ABS Limit [-32000][32000]
Auto Change Lot [Cancel] Compensated Limit [ ]
Bottle [Cancel]

Special Wash Programming
Cell Wash
[1] [Lambda] [00402] [350] - [00402] [350]

8.4.2 Parameters (Hitachi 912)

Application - Analyze

Select Test: [Lambda] Analyzer Cycle TIme: [10 sec] Diluent: [00301] [0]
Test Name [Lambda] Assay/Time/Point [2 point end] [10] [7] [31] [0][0]
App Code [***] Wavelength (2nd/Primary): [ ] [600]

Sample Volume

	Class 1			Class 2		
Normal:	[20]	[5]	[140]	[1]	[0]	[0]
Decrease:	[2]	[5]	[158]	[1]	[0]	[0]
Increase:	[5]	[0]	[0]	[1]	[0]	[0]

Reagent

R1:	[150]	[0]	[*****]	[0]
R2:	[90]	[0]	[*****]	[0]
R3:	[0]	[0]	[*****]	[0]
R4:	[0]	[0]	[*****]	[0]

	Class 1	Class 2	Dec/In	
Abs Limit:	[32000]	[32000]	[Increase]	Twin Test: [ ]
Prozone Limit:	[32000]	[32000]	[Upper]	

Application – Calibration

Select Test: [Lambda]
Calibration Type: [Logit-Log (4p)] [ ]
Point: [6] Span Point: [6]
Weight: [0]

Auto-Calibration
Time Out Change Over
[ ] [ ] Lot: [ ] 3D Limit: [999.9]
[ ] [ ] Bottle: [ ] Duplicate Limit: [99] x [32000] Abs
[ ] [ ] Sensitivity Range: [-99999] - [99999]
[ ] [ ] St. Abs. Range: [-32000] - [32000]

Application - Range

Select Test: [Lambda] Report Name: [Lambda Free] Date Mode: [On Board]
Test Name: [Lambda] [ ] Control Interval [ ] Unit [mg/L]
Instrument Factor : (Y=aX+b) a= [1.0] b= [0.0]
App Code: [***]
Sample Type: [Ser/Pl]

Expected Range

Age		Male		Female		[ ]	Use Qualitative Tables	
[1]	[Years]	[-99999]	[99999]	[-99999]	[99999]	[1]	[ ]	[ ]
[50]	[Years]	[-99999]	[99999]	[-99999]	[99999]	[2]	[ ]	[ ]
		[-99999]	[99999]	[-99999]	[99999]	[3]	[ ]	[ ]
						[4]	[ ]	[ ]
						[5]	[ ]	[ ]
						[6]		[ ]

Default Age: [0 Years – 1 Years]
Default Sex: [Male] Repeat Range: [-99999] [99999]

Class 1 Technical Range: [STD2 CONC] – [STD6 CONC]
Class 2 Technical Range: [-99999] [99999]

Application - Others

Select Test: [Lambda]

Standard:	[1]	[2]	[3]	[4]	[5]	[6]
Calib. Code:	[***]	[***]	[***]	[***]	[***]	[***]
Concentration *(% of Cal value):*	[000]	[20%]	[50%]	[80%]	[160%]	[267%]
Position:	[**]	[**]	[**]	[**]	[**]	[**]
Sample Volume:	[5]	[5]	[10]	[20]	[40]	[50]
Diluted S. Volume:	[0]	[5]	[5]	[5]	[5]	[5]
Diluent Volume:	[0]	[195]	[150]	[180]	[160]	[100]

Special Wash – Edit Cell

Test: [Lambda]

R1:		R2:	
Bottle Code:	[402]	Bottle Code:	[402]
Volume:	[350]	Volume:	[350]

8.4.3 Parameters (Hitachi 917)

Application - Analyze

Select Test: [Lambda]
Assay/Time/Point [2 point end] [10] [7] [34] [0] [0]
Wavelength (2nd/Primary): [ ] [600]

Sample Volume

Normal:	[20]	[5]	[140]
Decrease:	[2]	[5]	[158]
Increase:	[5]	[0]	[0]

Reagent

R1:	[150]	[0]	[*****]	[0]	Timing	
R2:	[90]	[0]	[*****]	[0]	[ ]	
R3:	[0]	[0]	[*****]	[0]	[ ]	
R4:	[0]	[0]	[*****]	[0]		

Diluent
[ ] Water
[●] Diluent [951] [00]

Abs Limit: [32000] [Increase]
Prozone Limit: [32000] [0] [0] [0] [0] [Upper]

Cell Detergent: [Detergent 1]
Twin Test: [ ]

*Open channels on the Roche Hitachi 917 can be programmed to use Roche Saline as the sample diluent. The 'Diluent' parameter found at the Maint/Utility–Application–Analyze page of the appropriate assay should be entered as '00951', the reagent code for a bottle of Roche Saline.

Application – Calibration

Calibration Type: [Logit-Log (4p)] Auto-Calibration
Point: [6] Time Out
Span Point: [6] [ ] Blank [ ]
Weight: [0] [ ] Span [ ]
[ ] 2 Point [ ]
[ ] End [ ]
SD Limit: [999.9] Change Over
Duplicate Limit: [99] % [32000] Abs Module [ ]
Sensitivity Range: [-99999] - [99999] Lot [ ]
S1. Abs. Range: [-32000] - [32000] Bottle [ ]

Application - Range

App Code: [***] Expected Range
Unit: [mg/L] Male
Report Name: [Lambda Free] [100] [Year] [-99999] [99999]
Data Mode: [Active] [100] [Year] [-99999] [99999]
Technical Limit: [STD2 CONC] [STD6 CONC] [-99999] [99999]
Repeat Limit: [-99999] - [99999] Female
[ ] Control Time Interval [ ] [100] [Year] [-99999] [99999]
[ ] Qualitative [100] [Year] [-99999] [99999]
[1] [ ] [ ] [-99999] [99999]
[2] [ ] [ ] Default
[3] [ ] [ ] Sex:
[4] [ ] [ ] [●] Male [ ] Female
[5] [ ] [ ] Range:
[6] [ ] [ ] [ ] Range 1 [ ] Range 2 [ ] Range 3 [ ]

Application - Others

Standard:	[1]	[2]	[3]	[4]	[5]	[6]
Calib. Code:	[***]	[***]	[***]	[***]	[***]	[***]
Concentration *(% of Cal value):*	[000]	[20%]	[50%]	[80%]	[160%]	[267%]
Rack No. Position:	[**]	[**]	[**]	[**]	[**]	[**]
Sample Volume:	[5]	[5]	[10]	[20]	[20]	[20]
Diluted S. Volume:	[0]	[5]	[5]	[5]	[5]	[5]
Diluent Volume:	[0]	[195]	[150]	[180]	[80]	[40]

Special Wash – Edit Cell

	R1		R2	
Test	Type	Volume	Type	Volume
[Lambda]	[D1]	[270]	[D1]	[270]

8.4.4 Parameters (Modular P)

Application - Analyze

Select Test: [Lambda]
Assay/Time/Point [2 point end] [10] [7] [34] [0] [0]
Wavelength (2nd/Primary): [ ] [600]

Sample Volume Reagent

Normal:	[20] [5]	[140]	R1:	[150] [0]	[*****] [0]	Timing	
Decrease:	[2] [5]	[158]	R2:	[90] [0]	[*****] [0]	[ ]	
Increase:	[5] [0]	[0]	R3:	[0] [0]	[*****] [0]	[ ]	
			R4:	[0] [0]	[*****] [0]		

Diluent
[ ] Water
[●] Diluent [311] *

Abs Limit: [32000] [Increase]
Prozone Limit: [32000] [0] [0] [0] [0] [Upper]
Cell Detergent: [Detergent 1]
Twin Test: [ ]

Application – Calibration

Calibration Type: [Logit-Log (4p)] Auto-Calibration
Point: [6] Time Out
Span Point: [6] [ ] Blank [ ]
Weight: [0] [ ] Span [ ]
[ ] 2 Point [ ]
[ ] End [ ]
SD Limit: [999.9] Change Over
Duplicate Limit: [99] % [32000] Abs Module [ ]
Sensitivity Range: [-99999] - [99999] Lot [ ]
S1. Abs. Range: [-32000] - [32000] Bottle [ ]

Application - Range

App Code: [***] Expected Range
Unit: [mg/L] Male
Report Name: [Lambda Free] [100] [Year] [-99999] [99999]
Data Mode: [Active] [100] [Year] [-99999] [99999]
Technical Limit: [STD2 CONC] [STD6 CONC] [-99999] [99999]
Repeat Limit: [-99999] - [99999] Female
[ ] Control Time Interval [ ] [100] [Year] [-99999] [99999]
[ ] Qualitative [100] [Year] [-99999] [99999]
[1] [ ] [ ] [-99999] [99999]
[2] [ ] [ ] Default
[3] [ ] [ ] Sex:
[4] [ ] [ ] [●] Male [ ] Female
[5] [ ] [ ] Range:
[6] [ ] [ ] [ ] Range 1 [ ] Range 2 [ ] Range 3 [ ]

Application - Others

Standard:	[1]	[2]	[3]	[4]	[5]	[6]
Calib. Code:	[***]	[***]	[***]	[***]	[***]	[***]
Concentration *(% of Cal value):*	[000]	[20%]	[50%]	[80%]	[160%]	[267%]
Rack No. Position:	[**]	[**]	[**]	[**]	[**]	[**]
Sample Volume:	[5]	[5]	[10]	[20]	[20]	[20]
Diluted S. Volume:	[0]	[5]	[5]	[5]	[5]	[5]
Diluent Volume:	[0]	[195]	[150]	[180]	[80]	[40]

Special Wash – Edit Cell

	R1		R2	
Test	Type	Volume	Type	Volume
[Lambda]	[D1]	[270]	[D1]	[270]

*Open channels on the Modular P will not use an onboard Roche Saline as a diluent. An additional bottle of saline must be manually registered to the test channel (e.g. FKAP) as a 'DIL' reagent. Remove the label from a Roche Saline bottle and replace with an open channel bottle label. The saline will need to be manually assigned to the test channel with a 'DIL' reagent type and positioned in the outer ring of the reagent carousel e.g. position 1-8. ONLY after manual registration should you place the diluent and other reagents on board the instrument. Then perform a Reagent Level Registration. This new diluent may be used for another open channel assay by using the same Diluent Bottle Code (311-400 or 901-910) on the appropriate Utility-Application-Analyze page of the parameters.

NB: Users must enter the Technical Limit of each new kit as specified on the accompanying batch-specific Product Data Sheet, SIN061.DS.

NB: Users should only use exact volumes of standard and control materials as detailed below:

- **Use 500µL of the Lambda free standard per calibration in a sample cup.**
- **Use 100µL of the Lambda free control per test in a sample cup.**
- **Use 100µL of the Lambda Free High Control per test in a sample cup.**

8.4.5 Measuring range

All samples must be first assayed at the standard 1/8 sample dilution, giving an approximate measuring range of 5.6 – 74.8mg/L. This enables a sensitivity of 0.7mg/L on neat serum samples. The upper limit of the measuring range using a sample dilution of 1/80 is 748mg/L. For samples measuring over this limit the following series of manual pre-dilutions should be used to minimise test usage:

Overall dilution	Analyser dilution	Manual pre-dilution	Approximate range (mg/L)
1/1	1/1	-	0.7 – 9.35
1/8	1/8	-	5.6 – 74.8
1/80	1/80	-	56 – 748
1/800	1/8	1/100*	560 – 7480
1/8000	1/80	1/100*	5600 - 74800

* 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.

9 QUALITY CONTROL

The controls provided should be included in all assay runs. The lambda free concentration is stated on the accompanying Product Data sheet (SIN061.DS). Results obtained during the run should only be accepted if the control results obtained are within ±20% of the concentration(s) stated.
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 supplier.

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 **Antigen excess**: A small proportion of patient samples containing high concentrations of free kappa or free lambda can give a falsely low result for the "involved" light chain due to antigen excess. The amino acid composition of the light chain produced by an individual B cell clone will influence the level at which a sample may show antigen excess with the **Freelite** assay. In almost every case the concentration of the involved light chain will still be above the quoted normal range (3.30-19.40mg/L for free kappa and 5.71-26.30mg/L for free lambda) and/or the opposite light chain concentration will be below the quoted range and/or the free kappa/free lambda ratio will be outside the quoted range (0.26-1.65). Samples should be tested at both the initial dilution and with a 1/100 manual predilution (see section 8.4.5) in order to detect antigen excess if any of the following conditions are met:

a) sample shows either a free light chain concentration or a free kappa/free lambda ratio outside of the quoted range,
b) sample is from a patient that has previously demonstrated antigen excess,
or
c) sample result does not agree with other clinical or laboratory findings.

10.4 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 10.3 above) then further investigation by other laboratory methods (immunofixation and serum protein electrophoresis).

10.5 The nature of monoclonal proteins can cause a non-linear response in immunoassays, potentially leading to inconsistent results; this can be prevented by always assaying the samples in the sequence 1/8, 1/80, 1/800, 1/8000 (see Section 8.4.5). Omitting a dilution step or using alternative dilutions must be avoided.

10.6 Due to the highly variable nature of monoclonal proteins, different reagent batches may react slightly differently to the FLC 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.

10.7 Customers should be aware that use of the NaOHD solution as both a special wash and regular washing fluid is essential in order to prevent cuvette fogging.

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 Adult serum ranges

282 normal subjects aged from 20 to 90 years were assayed using 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

In order to demonstrate equivalence of the normal range obtained with the BNII and Hitachi assays we have assayed on 100 normal samples from normal UK donors aged from 20 to 60 years and 54 disease state sera with both the BNII and Hitachi **Freelite** assays. The results of regression analysis are as follows: for the kappa assay, *y=0.94x + 2.85, r=0.96*, and for the lambda assay *y=0.99x + 0.46, r=0.99* (y = Hitachi value, x = BNII value). This demonstrates that the more extensive data generated at the Mayo Clinic is applicable to the Hitachi assays.

**BN™II is a trademark of Siemens Healthcare Diagnostics Inc.*

11.2 Normal urine results

Urinary free light chain concentrations were measured from samples provided by 58 healthy adult donors. For both free kappa and free lambda measurements a number of samples ran below the measuring range of the assay. A total available range has been quoted.

Normal adult urine	Percentage of samples below detection limit (%)	Total range (mg/L)
Free Kappa	10	<0.78 - 13.48
Free Lambda	62	<2.22 - 5.9

12 PERFORMANCE CHARACTERISTICS

12.1 Within-run precision

Three serum preparations containing different levels of free lambda were assayed. Each value given was calculated from 10 measurements made on the same assay run. All concentrations are in mg/L.

	Serum 1	Serum 2	Serum 3
Mean	10.7	42.8	57.4
CV%	3.67	2.55	3.46

12.2 Between-run precision

Three serum preparations containing different levels of free lambda were assayed on 10 separate assay runs using kits from a single batch. All concentrations are in mg/L.

	Serum 1	Serum 2	Serum 3
Mean	11.9	29.1	55.4
CV%	9.50	6.84	6.31

12.3 Linearity

The linearity of this assay has been confirmed using a serially diluted serum sample, which gave a regression plot of *y = 1.01x – 0.01 (mg/L), r = 1.00*. (y = measured free lambda concentration, x = theoretical concentration).

12.4 Interference

Minimal assay interference by 200mg/L bilirubin (0.32%), 5g/L haemoglobin (0.44%) and 2.5% chyle (-1.2%) has been demonstrated using a 34mg/L free lambda control serum.

12.5 Comparison

Sera: 54 sera (10 normal, 44 from known multiple myeloma or amyloid patients) were assayed by the **Freelite** BNII kappa and lambda kits and on three commercially-available immunofixation electrophoresis (IFE) kits. The clinical samples were assayed at a major independent reference centre in the USA.

	Freelite results				IFE Results
	Free Kappa	Free Lambda	Free κ/λ ratio	Summary	
Normal sera (10)	10 normal	10 normal	9 normal 1 borderline high	10 normal	10 normal
Myeloma/ amyloid sera (24)	24 high	12 normal 12 low	24 high	24 monoclonal kappa	19-24* show monoclonal band
Myeloma/ amyloid sera (20)	10 normal 10 low	15 high 5 normal	20 low	20 monoclonal lambda	12-14* show monoclonal band

**Method dependent.*

All abnormal serum light chain concentrations were detected by the **Freelite** assays, whereas some were missed by the less-sensitive IFE methods.

Urines: 28 urines (9 normal, 19 from known/suspected myeloma patients) were assayed by the **Freelite** kappa and lambda kits and on a commercially-available immunofixation electrophoresis (IFE) method.

	Freelite results				IFE Results
	Free Kappa	Free Lambda	Free κ/λ ratio	Summary	
Normal urine (9)	7 normal 2 borderline high	4 normal 5 borderline high	8 normal 1 borderline high	9 normal	9 normal
Kappa myeloma urine (9)	9 high	4 high 5 borderline high	8 high 1 normal	9 monoclonal kappa	9 show monoclonal band(s)
Lambda myeloma urine(10)	7 high 3 normal	10 high	10 low	10 monoclonal lambda	10 show monoclonal band

All abnormal urine light chain concentrations were detected by both the **Freelite** and IFE assays.

13 BIBLIOGRAPHY

1. Cole PW, Durie BGM, Salmon SE. (1978) Immunoquantitation of free light chain immunoglobulins: Application in multiple myeloma. J. Immunol. Meth. **19**: 341-349.
2. Pescali E, Pezozoli A. (1988) The clinical spectrum of pure Bence-Jones proteinuria. Cancer **61**: 2408-2415.
3. Solling K, Solling J, Romer FK. (1981) Free light chains of immunoglobulins in serum from patients with rheumatoid arthritis, sarcoidosis, chronic infections and pulmonary cancer. Acta. Med. Scand. **209**: 473-477.
4. Drayson MT, Tang LX, Drew R, Mead GP, Carr-Smith HD and Bradwell AR. (2001). Serum free light chain measurements for identifying and monitoring patients with non-secretory multiple myeloma. Blood **97**: 2900-2902.
5. Bradwell AR, Carr-Smith HD, Mead GP, Tang LX, Showell PJ, Drayson MT and Drew RL (2001). Highly sensitive, automated immunoassay for immunoglobulin free light chains in serum and urine. Clin. Chem. **47**: 4, 673-680.
6. Tang LX, Showell P, Carr-Smith HD, Mead GP, Drew R and Bradwell AR (2000). Evaluation of $F(ab')_2$-based latex-enhanced nephelometric reagents for free immunoglobulin light chains on the Behring Nephelometer™ II. Clin. Chem **46**:6, Suppl. 2000, 705, pA181.
7. Bradwell AR, Carr-Smith HD, Mead GP, Harvey TC and Drayson MT. (2003) Serum test for assessment of patients with Bence Jones myeloma. Lancet **361**: 489-491.
8. Abraham RS, Katzman JA, Clark RJ, Bradwell AR, Kyle RA and Gertz MA (2003). Quantitative Analysis of Serum Free Light Chains: A new marker for the diagnostic evaluation of primary systemic amyloidosis. Am. J. Clin. Pathol. **119**: (2): 274 – 278.
9. Lachmann HJ, Gallimore JR, Gillmore JD, Carr-Smith HD, Bradwell AR, Pepys MB and Hawkins PN (2003). Outcome in systemic AL amyloidosis in relation to changes in concentration of circulating immunoglobulin free light chains following chemotherapy. Brit. J.Haem. **122**: 78-84.
10. Bradwell AR, Carr-Smith HD, Mead GP and Drayson MT (2002) Serum free light chain immunoassays and their clinical application. Clinical and Applied Immunology Reviews **3**: 17 – 33.
11. Katzmann JA, Clark RJ, Abraham RS, Bryant S, Lymp JF, Bradwell, AR and Kyle RA. (2002) Serum reference intervals and diagnostic ranges for free kappa and free lambda immunoglobulin light chains: relative sensitivity for detection of monoclonal light chains. Clin. Chem. **48**: 1437-1444.
12. Bradwell AR (2009). Serum Free Light Chain Analysis, 5th Edition. Publ. The Binding Site Ltd, Birmingham, UK.
13. Mead GP, Carr-Smith HD, Drayson MT, Morgan GJ, Child JA and Bradwell AR (2004). Serum free light chains for monitoring multiple myeloma. Brit. J. Heamatol. **126**, 348-354