Freelite™ Human Kappa Free kit
For use on the Roche cobas® c systems

For *in-vitro* diagnostic use

Product Code: LK016.CB

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FDA (USA) Information
Analyte Name Kappa Light Chains
Complexity Cat. Moderate

CE

1 INTENDED USE

This kit is intended for the quantitation of kappa free light chains in serum on the c501 module of the Roche cobas® c6000 series. 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 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 (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 [1-11].

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 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 polyclonal kappa 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.

**ProClin™ is a trademark of Rohm and Haas Corp., Philadelphia, PA.*

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 *Human Kappa Free Reagent* and *Kappa Free Supplementary Reagent* may be stored in the C-pack for up to 3 months on the analyser after opening. The standard and controls may be stored at 2-8°C for up to 3 months after opening providing precautions to prevent evaporation and contamination are taken.

7 SPECIMEN COLLECTION AND PREPARATION

Use fresh or deep frozen serum samples. Serum should be obtained by venipuncture, allowed to clot and the serum separated as soon as possible to prevent hemolysis. 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 samples, samples containing particulate matter and lipaemic or haemolysed 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 the Binding Site's **Freelite** Lambda Free kit (LK018.CB).

8.1 Materials provided

8.1.1 1 x 100 tests *Human Kappa Free Reagent* (R2)
8.1.2 1 x 100 tests *Human Kappa Free Supplementary Reagent* (R1)
8.1.3 2 x 1.5mL *Human Kappa Free Standard*
8.1.4 1 x 1.0mL *Human Kappa Free Control*
8.1.5 1 x 1.0mL *Human Kappa Free High Control*
8.1.6 1 x Roche blue opening tool
8.1.7 1 x Roche barcoded C-pack cassette

8.2 Materials required but not provided

8.2.1 Equipment for collection and preparation of test samples e.g. sample tubes and cups (e.g. 2.5mL Hitachi cup), centrifuge, etc.
8.2.2 A fully operational and equipped cobas c analyser.
8.2.3 Special wash solution NAOHD C-pack, code 04489241190 and NACL diluent C-pack, code 04489357190 loaded onto the analyser.

8.3 Transferring R1 and R2 into C-pack *(see Figure 1)*

8.3.1 Transfer the Kappa Free Reagent (R2) into position B (on the right-hand side of the bottle as the barcode faces the user) and cap securely using the blue Roche opening tool.
8.3.2 Transfer the Kappa Free Supplementary Reagent (R1) into position A (middle bottle) and cap securely using Roche blue opening tool.
8.3.3 Leave position C empty and without cap.

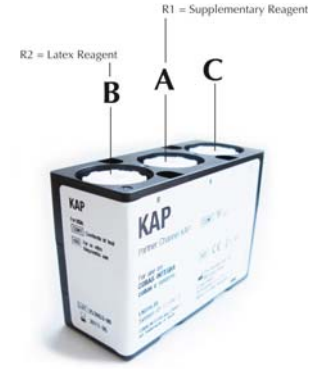

Figure 1: Transfer of reagents to C-pack.

8.4 Test procedure

The user should be familiar with the operation of the cobas c systems analyser before attempting to carry out the test procedures. Ensure the "KAP" application has been downloaded from cobas® Link. This is done in Utility > Application and then by selecting the Download button. Next, either select the Application code, 609, or Application name, KAP. Click on the Search button, tick the box on the link and Download. The KAP application should now appear in the test menu.

The Human Kappa Free Standard is available for download from cobas® Link. This is done in Calibration > Install and then by selecting the Download button. Click on CALIBRATOR NAME and select CAKAP. Click on the Search button, tick the selection checkbox and Download.

The Human Kappa Free Control and Human Kappa Free High Control are **NOT** available for download via the cobas® Link and must be manually installed.

Complete **Freelite** kit implemetation instructions for the cobas c systems analysers are available. Please contact your local Binding Site distributor for further information

8.4.1 In order to avoid carry-over from other chemistries, an NAOHD reagent and sample probe wash must be carried out to remove any interfering substances that may affect the Kappa **Freelite** result. The cells also require a special wash using NAOHD. Each extra wash action can be achieved by selecting **Utility** > **Special Wash** and then clicking **Edit** next to each component. Enter the following details:

Reagent Probe

		From Reagent		To Reagent		Detergent	
No.	Probe	Test Name	Type	Test Name	Type	Type	Volume
1	R1	All Item	R1	KAP	R1	D1	180
2	R2	All Item	R2	KAP	R2	D1	80
3	R2	All Item	R3	KAP	R2	D1	80

Sample Probe

No.	Test Name	Type
1	KAP	1

Cell

		R1		R2	
No.	**Test Name**	**Type**	**Volume**	**Type**	**Volume**
*	KAP	D1	180	D1	180
*					

* User defined position

8.4.2 In order to run **Freelite** tests, the reagents must be placed in the supplied Roche C-pack cassette (as shown above in Figure 1). Each C-pack has a unique barcode identified by the analyser, which allows only 100 tests to be aspirated. The cassette must then be discarded.

8.4.3 Users must enter the **Technical Limit** of each new kit lot in **Utility** > **Application** > **Range** as specified on the accompanying batch-specific Product Data Sheet, SIN129.DS.

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

- **Use 500µL of the Kappa Free Standard per calibration in a sample cup.**
- **Use 100µL of the Kappa Free Control per test in a sample cup.**
- **Use 100µL of the Kappa Free High Control per test in a sample cup.**

8.5 Measuring range

All samples must be assayed first at the initial 1/5 sample dilution, giving an approximate measuring range for the kappa free assay of 3.7-56.2mg/L. This allows a sensitivity of 0.8mg/L with neat serum samples. For samples measuring over the upper limit of the curve with an instrument dilution of 1/50, the recommended dilution series must be used.

Overall dilution	Instrument dilution	Manual Pre-dilution	Approximate range (mg/L)
1/1	1/1	-	0.8 – 11.2
1/5	1/5	-	3.7 – 56.2
1/50	1/50	-	37 – 562
1/500	1/5	1/100*	370 – 5620
1/5000	1/50	1/100*	3700 - 56200

*Make a manual pre-dilution of 1/100 by taking 100µL of sample and add 900µL of normal physiological saline (0.9%) to achieve an initial 1/10 dilution. From this, take 100µL of this dilution and add 900µL of normal physiological saline (0.9%) 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 must be included in all assay runs. The kappa free concentration is stated on the accompanying Product Data sheet (SIN129.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 your local Binding Site distributor.

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 8.5) 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 1/5, 1/50, 1/500, 1/5000 (see Section 8.5). 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.

10.8 Carry-over experiments have demonstrated that a number of other chemistries may interfere with Kappa free results. Therefore, users must run Kappa **Freelite** assays with the reagent and sample probe washes as detailed in section 8.4.1. Failure to do so may lead to elevation of the Kappa free result and distortion of the Kappa/Lambda ratio. The carry-over investigations have demonstrated that **Freelite** assays do **not** interfere with other chemistries. Please contact your local Binding Site distributor for further information.

11 EXPECTED VALUES

The ranges provided 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 21 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

In order to demonstrate equivalence of the normal range obtained with the BNII and Roche assays, 100 normal samples from UK donors aged from 20 to 60 years and 54 disease state samples were measured by both the BNII and Roche **Freelite** assays. The results of regression analysis are as follows: for the kappa assay, *y=0.94x + 2.85 (mg/L), r=0.96*, and for the lambda assay *y=0.99x + 0.46 (mg/L), r=0.99* (y = Roche value, x = BNII value). This demonstrates that the more extensive data generated at the Mayo Clinic is applicable to the Roche assays.
**BN™II is a trademark of Siemens Healthcare Diagnostics, Inc.*

12 PERFORMANCE CHARACTERISTICS

The following data was generated on a c501 analyser.

12.1 Within-run precision

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

	Kappa FLC		
	Mean (mg/L)	**SD**	**CV %**
Serum 1	5.58	0.08	1.4
Serum 2	18.47	0.46	2.5
Serum 3	41.08	0.68	1.7

12.2 Between-run precision

Three serum preparations containing different levels of free kappa were assayed on 3 separate assay runs using kits from a single batch. All concentrations are in mg/L.

	Kappa FLC		
	Mean (mg/L)	**SD**	**CV %**
Serum 1	5.79	0.42	7.2
Serum 2	19.76	1.38	7.0
Serum 3	41.35	1.21	2.6

12.3 Linearity

A linearity study was performed following NCCLS *Evaluation of the Linearity of Quantitative Measurement Procedures* (NCCLS Document EP6-A). One user assessed the linearity of a polyclonal sample diluted in a normal sample using one reagent lot on one analyser. This gave a regression plot of *y = 1.01x + 0.25 (mg/L), r = 1.00* (y = measured free kappa concentration, x = theoretical concentration).

12.4 Interference

Minimal assay interference by 150mg/L bilirubin (-9.7%), 4.8g/L haemoglobin (2.8%), 0.5% intralipid (4.4%) and 7,150FTU chyle (2.2%) was demonstrated using a 6.0mg/L free kappa control serum when assayed at the minimum sample dilution (1/1).

12.5 Lower Limit of Quantitation (0.8mg/L)

The limit of quantitation for this assay is 0.8mg/L, defined as the lowest point of the calibration curve at the lowest recommended sample dilution, i.e. neat (1/1).

12.6 Comparison

15 normal adult sera and 19 clinical adult sera (from known/suspected multiple myeloma and SLE patients) were tested on the **Freelite** c501 and **Freelite** Roche Modular P assays. Results were as follows:

	Kappa FLC
Sample range (mg/L)	6.4 – 1833
Passing Bablock regression	Y = 0.97x + 1.24 (mg/L)
Linear regression (R^2)	R^2 = 0.99

13 BIBLIOGRAPHY

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