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Freelite® Human Kappa Free kit
For use on the Roche Cobas Integra®

For *in vitro* diagnostic use

Product Code: LK016.RI

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FDA (USA) Information

Analyte Name	Kappa Light Chains
Complexity Cat.	Moderate

CE

Warning: The result of Kappa 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 kappa 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 Kappa 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 kappa free light chains in serum on the Roche Cobas Integra 400, 400*plus* and 800. 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 systemic lupus erythematosus [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 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 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.

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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 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° and can be used until the expiry date shown on the kit box label. DO NOT FREEZE. The latex and supplementary reagent may be stored in the C-pack for up to three months on the analyser after opening. The standard and controls may be stored at 2-8° 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 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° for up to 21 days, but for prolonged storage they should be kept frozen at -20° 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 Binding Site's **Freelite** Lambda Free kit (LK018.RI).

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 2mL *Human Kappa Free Standard*
8.1.4 2 x 1.5mL *Human Kappa Free Control*
8.1.5 2 x 1.5mL *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 Transferring R1 and R2 into C-pack *(see Figure 1).*

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

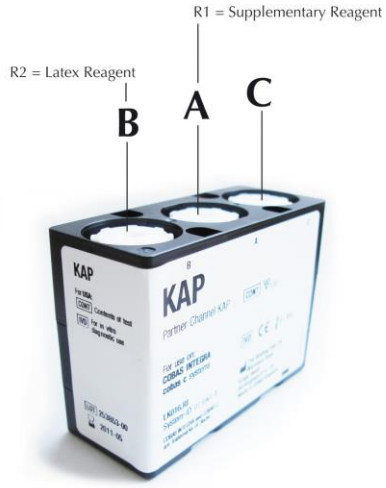

Figure 1: Transfer of reagents to C-pack.

NB: Integra 800 only. Once loaded onto the analyser, C-packs must remain on-board until all 100 tests have been used. Removal of C-packs before fully used will result in loss of remaining tests.

8.3 Materials required but not provided

8.3.1 Equipment for collection and preparation of test samples e.g. sample tubes (e.g. 650µL Cobas cup), centrifuge, etc.
8.3.2 A fully operational and equipped Integra 400/400*plus*/800 with NaCl Diluent 9% (Roche product code 20756350) loaded onto the ISE rack.
8.3.3 **Integra 800 only:** Cobas Integra Cleaner Cassette (CLEAN), Cat. No. 20764337, System ID 07 6433 7.

8.4 Test procedure

The user should be familiar with the operation of the INTEGRA 400/400*plus*/800 analyser before attempting to carry out the test procedures. Ensure the most current TASU (Test Application SW Update) has been uploaded.

8.4.1 **In order to avoid carry-over from other chemistries, Freelite tests must be batched together and run independently of other tests**. **A probe wash must be carried out prior to running in batch mode to remove any interfering substances that may affect the Freelite result**.

On the Integra 800
a) Select Configuration – Processing – Extra Wash Cycles
b) In the next empty space, tick Active and select All (tests) carries over to KAP and select the "CLEAN" cassette as cleaner.
c) In the comments, enter ALL/KAP/CLEAN

On the Integra 400/400*plus*
Carry out "beginning of day (BOD)" probe deproteinisation and "prime fluid system" actions.

8.4.2 In order to run **Freelite** tests, the reagents must be placed in the supplied Roche C-pack cassette (as shown above). 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 should only use exact volumes of standard and control materials as detailed below:

- **Use 450µL of the Kappa Free Standard per calibration in a sample cup.**
- **Use 150µL of the Kappa Free Control per test in a sample cup.**
- **Use 150µL of the Kappa Free High Control per test in a sample cup.**

Failure to aliquot sufficient calibrator volume into a sample cup will results in 12 tests being wasted.

Complete **Freelite** kit implemetation instructions for the Integra 400/400*plus*/800 analysers are available. Please contact your local Binding Site distributor for further information.

8.5 Measuring range

Integra 400 and 400*plus*: All samples must be assayed first at the Initial 1/10 assay dilution, giving an approximate measuring range of 2.9-127mg/L. Alternative reassay dilutions, known as Factor A, Factor B and Factor D, are also available; with Factor D, the assay sensitivity is reduced to 0.6mg/L. The upper limit of the measuring range using Factor A is 12700mg/L; for higher concentration samples make a 1/10 manual pre-dilution. See below for summary:

Factor	Integra stated dilution	Actual overall dilution	Manual pre-dilution	Approximate range (mg/L)
D	5	1:2	-	0.6 – 25.3
Initial	1:1	1:10	-	2.9 – 127
B	1:10	1:100	-	29 – 1270
A	1:100	1:1000	-	290 – 12700
A	1:100	1:10000	1/10*	2900 - 127000

*Make a manual pre-dilution of 1/10 by taking 100µL of sample and add 900µL normal physiological saline (0.9%). Present the 1/10 diluted sample for analysis using Factor A. Multiply the result x 10.

Integra 800: All samples must be assayed at the Initial 1/10 sample dilution, giving an approximate measuring range of 2.9-127mg/L. Alternative reassay dilutions known as "Dilute" and "Concentrate" are available. With "Concentrate" the assay sensitivity is reduced to 0.6mg/L. The upper limit of the measuring range using "Dilute" is 1270mg/L. For higher concentration samples make a 1/100 manual pre-dilution and present this for analysis at the default assay dilution.

Post-action	Integra stated dilution	Actual overall dilution	Manual pre-dilution	Approximate range (mg/L)
Concentrate	5	1:2	-	0.6 – 25.3
Initial	1:1	1:10	-	2.9 – 127
Dilute	1:10	1:100	-	29 – 1270
Manual dilution	1:1	1:1000	1/100**	290 – 12700
Manual dilution (automatic repeat)	1:10	1:10000	1/100**	2900 - 127000

**Make a manual 1/100 predilution by taking 100µL of sample and add 900µL normal physiological saline (0.9%) to achieve an initial 1/10 dilution. From this, take 100µL of this dilution and add 900µL of physiological saline (0.9%) to achieve a final 1/100 dilution. Present the 1/100 diluted sample for analysis. Multiply the result x 100.

8.6 Antigen Excess

All turbidimetric assays may 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 Integra monitors the initial reaction kinetics of each sample and compares the results to reaction limits set through testing of an extensive myeloma library. Samples detected as being in excess are flagged with "HIGH ACTIVITY" in the results and should be remeasured at a higher dilution to remove the antigen excess (see Section 8.5). On the Integra 400/400*plus* remeasure samples at the 1:10 non-standard dilution (Factor B) and then 1:100 non-standard dilution (Factor A) if required. On the Integra 800 remeasure samples using the Dilute reassay conditions and with a 1/100 manual predilution if required.

Important Note: A very small percentage of samples in antigen excess have normal reaction kinetics so will not prompt the "HIGH ACTIVITY" 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 sample dilution."

9 QUALITY CONTROL

The controls provided should be included in all assay runs. The kappa free concentration is stated on the accompanying Product Data sheet (SIN122.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 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. Possible interference due to the presence of rheumatoid factor can also occur (see Section 12.6)
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**: See section 8.6

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. Samples suspected to be in antigen excess which is not detected by the instrument should be tested by repeating at a higher dilution to preclude antigen excess (see section 8.6).This should be followed by further investigation using 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 avoided by always diluting the samples in the sequence 1:1, 1:10, 1:100 (Initial, Factor B, Factor A on the Integra 400/400*plus* or "Initial", "Dilute" on the Integra 800 – see Section 8.5). Omitting a dilution step or using alternative dilutions should 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 Carry-over experiments have demonstrated that a number of other chemistries may interfere with Kappa Free results when run in a random access mode. Therefore, users must run all **Freelite** assays in batch mode 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 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. (mg/L)	Median conc. (mg/L)	95 Percentile range (mg/L)
Free kappa	8.36	7.30	3.30 - 19.40
Free lambda	13.43	12.40	5.71 - 26.30
	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 Integra assays, 50 samples from normal UK donors aged from 20 to 60 years were assayed using both the BNII and Integra **Freelite** kits. Results are summarised under "Normal Sera" in Section 12.8.

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12 PERFORMANCE CHARACTERISTICS

A precision study was performed following NCCLS *Evaluation of Precision Performance of Clinical Chemistry Approved Guideline* (NCCLS Document EP5-A). The study was carried out on an Integra 400 over 21 working days, with two runs per day. One user assessed three different samples using three different reagent lots on one analyser.

12.1 Within-run precision

	Kappa FLC		
	Mean (mg/L)	**SD**	**CV %**
Serum 1	5.99	0.35	5.8
Serum 2	18.72	0.4	2.1
Serum 3	95.64	1.38	1.4

12.2 Between-run precision

	Kappa FLC		
	Mean (mg/L)	**SD**	**CV %**
Serum 1	5.99	0.16	2.7
Serum 2	18.72	0.51	2.7
Serum 3	95.64	1.68	1.8

12.3 Between-day precision

	Kappa FLC		
	Mean (mg/L)	**SD**	**CV %**
Serum 1	5.99	0.22	3.6
Serum 2	18.72	0.6	3.2
Serum 3	95.64	2.01	2.1

12.4 Total precision

	Kappa FLC		
	Mean (mg/L)	**SD**	**CV %**
Serum 1	5.99	0.44	7.4
Serum 2	18.72	0.88	4.7
Serum 3	95.64	2.97	3.1

12.5 Linearity

The linearity of this assay was confirmed using a serially diluted polyclonal serum sample, which gave a regression plot of *y = 1.003x – 0.937 (mg/L), r = 0.99* (y = measured free kappa concentration, x = theoretical concentration).

12.6 Interference

Minimal assay interference by 200mg/L bilirubin (-9.4%), 5.7g/L haemoglobin (8.2%) and 0.2% intralipid (1.3%) was demonstrated using a 7.1mg/L free kappa control serum. Slight interference (+15.1%) by 320 IU/mL rheumatoid factor has been demonstrated using a 15mg/L free kappa serum sample.

12.7 Analytical sensitivity

The analytical sensitivity of this assay (0.6mg/L) was confirmed by assaying ten replicates of two pooled human serum samples with free kappa concentrations equivalent to 140% and 200% of this value; the two sets of results did not overlap.

12.8 Comparison

50 normal adult sera and 82 clinical adult sera (from known/suspected multiple myeloma and systemic lupus erythematosus patients) were tested on the **Freelite** Integra and **Freelite** BNII assays. Results were as follows:

	Normal sera	Clinical sera
Range (mg/L)	5-24mg/L	0.4-1900mg/L
Passing Bablock regression	y=0.9282x + 1.506	y=1.07x – 0.19*
linear regression R^2	R^2 =0.9168	R^2 =0.9316*

* Two markedly high monoclonal values (5,000 and 12,000 mg/L) were excluded from the calculation.

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