

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY**

A. 510(k) Number:

k070900

B. Purpose for Submission:

New analyzer

C. Measurand:

Immunoglobulins, Kappa (κ) free light chains and Lambda (λ) free light chains

D. Type of Test:

Quantitative, Nephelometry or turbidimetry

E. Applicant:

The Binding Site, Ltd.

F. Proprietary and Established Names:

FREELITE™ Human Kappa Free Kit for use on Roche COBAS® INTEGRA
400/400*plus*

FREELITE™ Human Lambda Free Kit for use on Roche COBAS® INTEGRA
400/400*plus*

G. Regulatory Information:

1. Regulation section:

21 CFR § 866.5550, Immunoglobulin (light chain specific) immunological test system

2. Classification:

Class II

3. Product codes:

DFH - Kappa, antigen, antiserum, control

DEH - Lambda, antigen, antiserum, control

4. Panel:

Immunology (82)

H. Intended Use:

1. Intended use(s):

The FREELITE™ Human Kappa Free kit is intended for the quantitation of Kappa free light chains in serum on the Roche COBAS INTEGRA 400 and 400*plus*. Measurement of light chains aids in the diagnosis and monitoring of multiple myeloma, lymphocytic neoplasms, Waldenstrom's macroglobulinemia, amyloidosis, light chain deposition disease and connective tissue diseases such as systemic lupus erythematosus in conjunction with other laboratory and clinical findings.

The FREELITE™ Human Lambda Free kit is intended for the quantitation of Lambda free light chains in serum on the Roche COBAS INTEGRA 400 and 400*plus*. Measurement of light chains aids in the diagnosis and monitoring of multiple myeloma, lymphocytic neoplasms, Waldenstrom's macroglobulinemia, amyloidosis, light chain deposition disease and connective tissue diseases such as systemic lupus erythematosus in conjunction with other laboratory and clinical findings.

2. Indication(s) for use:
Same as Intended use.
3. Special conditions for use statement(s):
For prescription only.
4. Special instrument requirements:
Roche COBAS® INTEGRA 400/400*plus*

I. Device Description:

The FREELITE™ Human Kappa and Lambda Free kit contains polyclonal monospecific antibody coated onto polystyrene latex, a standard, two controls (high and low polyclonal kappa or lambda free light chain), and supplementary reagent.

J. Substantial Equivalence Information:

1. Predicate device name(s):
FREELITE™ Human Kappa Free Kit for use on Dade Behring Nephelometer™ II
FREELITE™ Human Lambda Free Kit for use on Dade Behring Nephelometer™ II
2. Predicate K number(s):
k010440 (Kappa)
k010441 (Lambda)
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Indication for Use	Measurement of light chains aids in the diagnosis and monitoring of multiple myeloma, lymphocytic neoplasms, Waldenstrom's macroglobulinemia, amyloidosis, light chain deposition disease and connective tissue diseases such as systemic lupus erythematosus in conjunction with other laboratory and clinical findings	Same
Controls	Human sera containing polyclonal free light chain, in stabilized liquid form, contain 0.099% sodium azide, 0.1% EACA, 0.01% benzamidine. Packaged in glass vials	Same
Stability/ storage of unopened reagent	2-8°C until expiry date	Same

Differences		
Item	Device	Predicate
Instrument	Roche COBAS INTEGRA 400 and 400 <i>plus</i>	Dade Behring Nephelometer II (BN™ II)
Technology	Turbidimetry	Nephelometry
Assay times	8.3 minutes	18 minutes

Differences		
Item	Device	Predicate
Cuvettes	Disposable	Washable
Sample matrix	Serum	Serum and urine
Sample dilution	1:10	1:100
Kappa or Lambda Latex Reagent	Supplied in Nalgene plastic bottles. Transferred to C-Pack prior to assay.	Supplied in Nalgene plastic bottles. No transfer prior to assay.
Supplementary Reagent	Type 116 Latex buffer containing Sodium Azide (NaN ₃) 0.099%. Type 265 PEG solution can be added for optimizing reaction if required. Supplied in Nalgene plastic bottles. Transferred to C-Pack prior to assay.	Type 49 Distilled water containing Sodium Azide (NaN ₃) 0.099%. Type 265 PEG solution can be added for optimizing reaction if required. Packaged in glass vials.
Opened reagent Storage/ stability	2-8°C up to 1 month	2-8°C up to 3 months
Measuring range	κ : 2.9 – 127 mg/L (1:10) λ : 5.2 – 139 mg/L (1:8)	κ : 5.9 – 190 mg/L (1:100) λ : 8.1 – 260 mg/L (1:100)
Sensitivity (serum)	κ : 0.6 mg/L (1:2) λ : 1.3 mg/L (1:2)	κ : 0.3 mg/L (1:5) λ : 0.4 mg/L (1:5)
Linearity	κ : $y = 1.003x - 0.937$, $r = 0.99$ λ : $y = 1.004x - 1.123$, $r = 1.0$	κ : $y = 0.98x + 0.83$, $r = 0.99$ λ : $y = 0.99x - 0.63$, $r = 1.0$

K. Standard/Guidance Document Referenced (if applicable):

CLSI (NCCLS) EP5-A: Evaluation of Precision Performance of Clinical Chemistry Approved Guideline.

L. Test Principle:

The concentration of a soluble antigen is nephelometrically or turbidimetrically measured by 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. The antibody in the cuvette is in excess so the amount of immune complex formed is proportional to the antigen concentration. In nephelometry the light scatter is monitored by measuring the light intensity at an angle away from incident light, whilst in turbidimetry light scatter is monitored by measuring the decrease in intensity of the incident beam of light. 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 nephelometric or turbidimetric assays can be increased by the use of particle enhancement. This entails linking the antibody to a suitably sized particle that increases the relative light-scattering signal of the antigen-antibody reaction.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

The study was carried out by testing three samples with different concentrations of kappa or lambda light chains using three different reagent lots on one analyzer. The study was performed over 21 working days, with two runs per day. Results are summarized below:

Kappa Precision	Within run		Between-run		Between-day		Total Precision	
	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Low (mean 5.99 mg/L)	0.35	5.8	0.16	2.7	0.22	3.6	0.44	7.4
Medium (mean 18.72 mg/L)	0.40	2.1	0.51	2.7	0.60	3.2	0.88	4.7
High (mean 95.64 mg/L)	1.38	1.4	1.68	1.8	2.01	2.1	2.97	3.1
Lambda Precision								
Lambda Precision	Within run		Between-run		Between-day		Total Precision	
	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Low (mean 7.72 mg/L)	0.17	2.3	0.19	2.5	0.29	3.7	0.39	5.0
Medium (mean 27.0 mg/L)	0.18	0.7	0.21	0.8	0.42	1.5	0.50	1.9
High (mean 99.2 mg/L)	0.72	0.7	0.73	0.7	1.60	1.6	1.90	1.9

b. *Linearity/assay reportable range:*

Linearity was confirmed using serial dilutions of polyclonal samples. The regression plot equations where y is the measured level of free chain concentration and x the theoretical concentration were:

$$y = 1.003x - 0.937 \text{ (mg/L), } r = 0.99 \text{ for } \kappa \text{ chains}$$

$$y = 1.004x - 1.123 \text{ (mg/L), } r = 1.00 \text{ for } \lambda \text{ chains}$$

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

No reference standards are available for these analytes. A calibrator and two controls are provided with each kit. No changes were made from previous cleared submission.

On-board stability studies were tested at 0, 4, 8, 13, 17, 24, 31, 38 day intervals. Stability results over the testing period meet the acceptance criteria of $\pm 20\%$ differences.

d. *Detection limit:*

Analytical sensitivity was determined by assaying ten replicates of two samples with concentrations close to the lowest calibration points. The analytical sensitivity claims were: 0.6 mg/L for kappa and 1.3 mg/L for lambda.

e. *Analytical specificity:*

Interference study: the table below shows common substances that could interfere with this method. Samples were run in triplicate. Minimum interferences were observed except for 15.5% difference with Rheumatoid factor on kappa antiserum. The package insert states the information below.

Interferent	Concentration	% Difference	
		κ (15 mg/L)	λ (18 mg/L)
Bilirubin	200.0 mg/L	-9.4	-1.7
Haemoglobin	5.7 g/L	8.2	3.8
Intralipid	0.2%	1.3	
	0.5%		-1.9
Rheumatoid factor	320.0 I.U.	15.5	
	480.0 I.U.		3.3

f. *Assay cut-off:*

Refer to Expected values.

2. Comparison studies:

a. *Method comparison with predicate device:*

A total of 132 serum samples were tested on the Freelite Kappa and Lambda Roche Cobas Integra 400/400*plus* and Freelite Kappa and Lambda Dade Behring BNII assays. Fifty (50) samples were normal adult sera and 82 samples were abnormal adult sera (58 known/suspected multiple myeloma (<80 mg/dL to >1000 mg/dL) and 24 systemic lupus erythematosus). The differences observed between the two assays were likely due to different test parameters and kinetics of reaction. The data including regression analysis results were as follows:

Samples	Kappa	Lambda
Normal Sera (n = 50)		
Range (mg/L)	5-24 mg/L	3-19 mg/L
Deming regression	$y=0.932x + 1.472$	$y=1.08x - 1.09$
R^2	$R^2=0.9168$	$R^2=0.9351$
Clinical Sera (n = 82)		
Range (mg/L)	0.4-1,900 mg/L	1-18,700 mg/L
Deming regression	$y=0.90x + 23.54^*$	$y=0.892x + 50.06$
R^2	0.9316	0.9673

*Two markedly high monoclonal values (5000 and 120000 mg/L) were excluded from calculation.

b. *Matrix comparison:*

Not applicable,

3. Clinical studies:

a. *Clinical Sensitivity:*

Not applicable.

b. *Clinical specificity:*

Not applicable.

c. Other clinical supportive data (when a. and b. are not applicable):

Not applicable.

4. Clinical cut-off:

Not applicable.

5. Expected values/Reference range:

The reference range table provided below were from 282 normal subjects aged

from 20 – 90 years which were assayed using Binding Site FREELITE Kappa and Lambda Free kits on BN II™ (Katzmann, JA et al. 2002 Clin Chem 48: 1437 – 1444).

Normal adult serum	Mean conc.	Median conc.	95 th 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 addition, the package insert includes the following statements:

- 1.) “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.”

- 2.) “In order to demonstrate equivalence of the normal range obtained with the BNII and INTEGRA assays, 50 normal samples from UK donors aged 20 – 60 years were assayed on both BN and INTEGRA FREELITE kits”. Results of regression analysis were:
 Kappa assay
 $(\text{INTEGRA}) = 0.932(\text{BNII}) + 1.472, R^2 = 0.9168;$
 Lambda assay
 $(\text{INTEGRA}) = 1.08(\text{BNII}) - 1.09, R^2 = 0.9351$

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.