510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K211648

B Applicant

Diazyme Laboratories Inc.

C Proprietary and Established Names

Diazyme Human Kappa (κ) Free Light Chain Assay
Diazyme Human Lambda (λ) Free Light Chain Assay

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
DFH, DEH	Class II	21 CFR 866.5550 - Immunoglobulin (Light Chain Specific) Immunological Test System	IM - Immunology

II Submission/Device Overview:

A Purpose for Submission:

Modification of previously cleared devices – revision of Limit of Quantification (LoQ) claim

B Measurand:

Kappa Free Light Chain (FLC)
Lambda Free Light Chain (FLC)

C Type of Test:

Latex particle enhanced immunoturbidimetric, quantitative

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The Diazyme Human Kappa Free Light Chain Assay is intended as a latex particle enhanced immunoturbidimetric assay for the quantitative determination of Kappa Free Light Chain (FLC) concentration in serum on validated analyzers. The measurement of Kappa FLC in conjunction with Lambda FLC aids in the diagnosis and monitoring of multiple myeloma in conjunction with other laboratory and clinical findings. For in-vitro diagnostic use only.

The Diazyme Human Lambda Free Light Chain Assay is intended as a latex particle enhanced immunoturbidimetric assay for the quantitative determination of Lambda Free Light Chain (FLC) concentration in serum on validated analyzers. The measurement of Lambda FLC in conjunction with Kappa FLC aids in the diagnosis and monitoring of multiple myeloma in conjunction with other laboratory and clinical findings. For in-vitro diagnostic use only.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

Warning: The result of the Kappa FLC or Lambda FLC in a given specimen determined with assays and/or instrument platforms 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 FLC or Lambda FLC 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 the Kappa FLC and Lambda FLC 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.

D Special Instrument Requirements:

Roche cobas c 501

IV Device/System Characteristics:

A Device Description:

The Diazyme Human Kappa FLC Assay is comprised of the following reagents:

- Reagent R1: Tris buffer solution
- Reagent R2: Latex particles coated with polyclonal rabbit and goat anti-human kappa FLC antibodies

Material required but not provided:

- Calibrators: A five-level set in liquid form (5 x 1 mL) with target concentrations: 10, 20, 40, 80, 160 mg/L, ready to use
- Controls Set: Two levels (15 mg/L and 30 mg/L), serum based, liquid form

The Diazyme Human Lambda FLC Assay is comprised of the following reagents:

- Reagent R1: Tris buffer solution
- Reagent R2: Latex particles coated with polyclonal rabbit anti-human lambda FLC antibodies

Material required but not provided:

- Calibrators: A five-level set in liquid form (5 x 1 mL) with target concentrations: 10, 25, 55, 110, 220 mg/L, ready to use
- Control Set: Two levels (15 mg/L and 30 mg/L), serum based, liquid form

B Principle of Operation:

The Diazyme Human Kappa FLC Assay or the Diazyme Human Lambda FLC Assay is based on a latex enhanced immunoturbidimetric assay. Kappa FLC or Lambda FLC in the sample binds to specific anti-Kappa FLC or anti-Lambda FLC antibody, which is coated on latex particles, and causes agglutination. The degree of the turbidity caused by agglutination can be measured optically and is proportional to the amount of Kappa FLC or Lambda FLC in the sample. The instrument calculates the Kappa FLC or Lambda FLC concentration by interpolation of obtained signal of a 6-point calibration curve prepared from calibrators of known concentrations.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Diazyme Human Kappa (κ) Free Light Chain Assay
Diazyme Human Lambda (λ) Free Light Chain Assay

B Predicate 510(k) Number(s):

K220001

C Comparison with Predicate(s):

Device & Predicate Device(s):	K211648	K220001
Device Trade Name	Diazyme Human Kappa FLC Assay Diazyme Human Lambda FLC Assay	Same
General Device Characteristic Similarities		
Intended Use/ Indications For Use	The Diazyme Human Kappa Free Light Chain Assay is intended as a latex particle enhanced immunoturbidimetric assay for the quantitative determination of Kappa Free Light Chain (FLC) concentration in serum on validated analyzers. The measurement of Kappa FLC in conjunction with Lambda FLC aids in the diagnosis and monitoring of multiple myeloma in conjunction with other laboratory and clinical findings. For in-vitro diagnostic use only. The Diazyme Human Lambda Free Light Chain Assay is intended as a latex particle enhanced immunoturbidimetric assay for the quantitative determination of Lambda Free Light Chain (FLC) concentration in serum on validated analyzers. The measurement of Lambda FLC in conjunction with Kappa FLC aids in the diagnosis and monitoring of multiple myeloma in conjunction with other laboratory and clinical findings. For in-vitro diagnostic use only.	Same
Analyte	Kappa FLC Lambda FLC	Same
Measurement	Quantitative	Same
Detection Method	Latex particle immunoturbidimetric	Same
Instrument	Roche cobas c 501	Same
Sample type	Serum	Same
Capture Antibody	Polyclonal rabbit and goat anti-Kappa / anti-Lambda FLC antibodies coated on polystyrene latex microparticles.	Same
Reference range	Kappa: 2.37 – 20.73 mg/L Lambda: 4.23 – 27.69 mg/L K/L Ratio: 0.22 – 1.74	Same

General Device Characteristic Differences		
Analytical Measuring Interval	Kappa FLC: 2.9 – 150 mg/L 2.9 – 3,000 mg/L (extended)	Kappa FLC: 4.5 – 150 mg/L 4.5 – 3,000 mg/L (extended)
	Lambda FLC: 3.5 – 200 mg/L 3.5 – 4,000 mg/L (extended)	Lambda FLC: 6.1 – 200 mg/L 6.1 – 4,000 mg/L (extended)

VI Standards/Guidance Documents Referenced:

- CLSI EP06, 2nd Edition, Evaluation of Linearity of Quantitative Measurement Procedures
- CLSI EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

   Refer to K153394

2. Linearity:

   The linearity of the Diazyme Human Kappa FLC Assay and the Diazyme Human Lambda FLC Assay across their respective analytical measuring interval (AMI) was evaluated in accordance with CLSI guidelines EP06 2nd Edition. For each assay, a dilution panel of 11 levels with measurand concentrations across AMI was prepared by combining a 'high serum sample pool" and a 'low serum sample pool'. The 'high serum sample pool' was the pooled unmodified (native) patient serum specimens with analyte concentration above the 'upper limit of the linearity interval' (ULLI). The 'low serum sample pool' was analyte depleted sample prepared from the pooled native samples and with the analyte concentration below the 'lower limit of the linearity interval' (LLLI). For each assay, two dilution series were prepared, one covering the entire AMI (full range), the other covering the reference range (low range) of the assay. The dilution samples were tested on Roche cobas c 501 using two reagent lots for each lot, each dilution sample panels were tested in three replicates for full range series and in five replicates for low range series.

   Diazyme Human Kappa FLC Assay

   The data of the linearity study on Roche cobas c 501 with a representative lot for the dilution series covering the entire AMI are summarized in the table below:

Diazyme Human Kappa FLC Assay				
Dilution Level	**Measured Mean Value (mg/L)**	**Expected Value (mg/L)**	**Predicted Value* (mg/L)**	**% Deviation****
1	2.8	2.8	2.8	0.3%
2	21.1	20	19.9	5.8%
3	37.6	37.3	37.2	1.2%
4	53.6	54.5	54.4	-1.3%
5	70.6	71.8	71.6	-1.4%
6	88.3	89	88.8	-0.5%
7	107.6	106.3	106.0	1.5%
8	126.2	123.5	123.2	2.5%
9	144.1	140.7	140.3	2.7%
10	159.6	158.0	157.6	1.3%
11	175.2	175.2	174.7	0.3%

* Predicted Value, Y =0.997 x Expected

** % Deviation = 100 [(Measured –Predicted)/Predicted]

The linear regression was analyzed based on the data from each of two lots and the results are summarized in the following table:

Linearity of Diazyme Human Kappa FLC on Roche cobas c 501				
	Range (mg/L)	**Slope (95% CI)**	**Intercept (95% CI)**	**R^2**
Lot 1	1.9 – 22.6	1.009 (0.992; 1.025)	-0.34 (-0.57; -0.11)	0.9995
	2.8 – 175.2	0.999 (0.988; 1.010)	-0.13 (-0.80; 0.54)	0.9995
Lot 2	1.2 – 22.6	1.009 (0.991; 1.026)	-0.07 (-0.31; 0.18)	0.9998
	1.8 – 168.7	1.023 (1.018; 1.028)	0.89 (0.50; 1.29)	0.9992

The results support the linearity of the claimed AMI of 2.9 – 150 mg/L for the Diazyme Human Kappa FLC Assay.

Diazyme Human Lambda FLC Assay

The data of the linearity study on Roche cobas c 501 with a representative lot for the dilution series covering the entire AMI are summarized in the table below:

Diazyme Human Lambda FLC Assay				
Dilution Level	**Measured Mean Value (mg/L)**	**Expected Value (mg/L)**	**Predicted Value* (mg/L)**	**% Deviation****
1	3.0	3.0	3.0	-0.4%
2	23.0	24.7	24.8	-7.1%
3	45.6	46.3	46.5	-1.8%
4	69.2	68.0	68.3	1.3%
5	89.6	89.7	90.0	-0.5%
6	112.9	111.3	111.7	1.0%
7	135.4	133	133.5	1.4%
8	156.9	154.6	155.2	1.1%
9	178.4	176.3	177.0	0.8%
10	199.1	198	198.8	0.2%
11	219.6	219.6	220.4	-0.4%

* Predicted Value, Y =1.004 x Expected

** % Deviation = 100 [(Measured –Predicted)/Predicted]

The linear regression was analyzed based on the data from each of two lots and the results are summarized in the following table:

Linearity of Diazyme Human Lambda FLC on Roche cobas c 501				
	Range (mg/L)	**Slope (95% CI)**	**Intercept (95% CI)**	**R^2**
Lot 1	2.7 – 33.8	0.989 (0.963; 1.016)	-0.50 (-1.06; 0.06)	0.9974
	3.0 – 219.6	1.010 (1.004; 1.017)	-0.38 (-1.21; 0.46)	0.9998
Lot 2	2.5 – 30.5	1.005 (0.990; 1.020)	-0.07 (-0.36; 0.21)	0.9999
	2.7 – 224.5	1.016 (1.008; 1.024)	-2.21 (-3.27; -1.16)	0.9996

The results support the linearity of the claimed AMI of 3.5 – 200 mg/L for the Diazyme Human Lambda FLC Assay.

Additionally, the linearity of the Diazyme Human Kappa FLC Assay and the Diazyme Human Lambda FLC Assay was validated following the same protocol described above using two reagent lots on the following instruments: Abbott Architect c8000, Beckman AU680, and Diazyme c270.

3. Analytical Specificity/Interference:

   Refer to K153394

4. Assay Reportable Range:

   Refer to K153394

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

Refer to K153394

6. Detection Capability:

CLSI guideline EP17-A2 were followed to determine the Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) for the Diazyme Human Kappa FLC Assay and the Diazyme Human Lambda FLC Assay.

LoB: Four blank samples were prepared from 'native samples' by immune-depleting the measurands. The Kappa FLC and Lambda FLC depleted samples were tested in replicates of five over the course of three days using two reagent lots on one Roche cobas c 501 analyzer. The LoB was calculated as the 95th percentile for each lot (a total of 60 replicates for each lot). The claimed LoB for each assay was established to be the highest LoB across the two lots.

LoD: Four low serum samples prepared from 'native samples' diluted with Kappa FLC and Lambda FLC depleted serum. The diluted samples were tested in replicates of five over the course of three days using two reagent lots on one Roche cobas c 501 analyzer (a total of 60 replicates for each lot). The parametric analysis resulted into: $LoD = LoB + 1.648 \times SD_L$ (SD_L= pooled SD across all low-level samples). The claimed LoD for the assay was the highest LoD of the two lots and instruments and defined as lowest concentration of analyte that can be detected consistently (in ≥ 95% of samples tested in routine clinical laboratory conditions).

LoQ: Six serum samples containing different levels of analyte targeted at around estimated LoQ were prepared by diluting 'native samples' with Kappa FLC and Lambda FLC depleted serum sample. The samples were tested in replicates of five over the course of three days using two reagent lots on one Roche cobas c 501 analyzer (a total of 90 replicates for each lot). The LoQ was defined to be the lowest concentration level that meets the within-laboratory imprecision of < 20% for each lot. The claimed LoQ for each assay was established as the greatest LoQ across the two lots and set as the LLLI.

The claimed LoB/LoD/LoQ for the Diazyme Human Kappa FLC and Lambda FLC Assay are as follows:

	LoB	**LoD**	**LoQ**
Diazyme Human Kappa FLC Assay	0.9 mg/L	1.8 mg/L	2.9 mg/L
Diazyme Human Lambda FLC Assay	1.6 mg/L	2.8 mg/L	3.5 mg/L

Additionally, the LoQ of each assay was verified on the Roche cobas c 501 instrument family including Roche cobas c 311, Roche cobas c 701, Roche cobas c 503 analyzer, and Abbott Architect c4000 using two native samples (diluted with analyte depleted serum to the target concentration of LoQ claims) in seven replicates, one reagent lot, over three days of testing on one instrument (with a total of 42 observations).

The LoB, LoD and LoQ study was also performed on the Abbott c8000, Beckman AU 680, and Diazyme c270 according to the protocol described above. The results support the claimed LoB, LoD, and LoQ of each assay using these instruments.

7. Assay Cut-Off:

   Refer to K153394 and K184338

B Comparison Studies:

1. Method Comparison with Predicate Device:

   Refer to K153394

2. Matrix Comparison:

   Not applicable

C Clinical Studies:

Refer to K153394 and K220001

D Clinical Cut-Off:

Refer to K153394 and K220001

E Expected Values/Reference Range:

Refer to K153394 and K184338

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

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