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Diazyme Human Kappa (κ) Free Light Chain Assay

The Diazyme Human Kappa (κ) Free Light Chain Assay is provided in bulk and the following kit configurations:

REF	Kit size
DZ169A-K	R1: 1 x 22 mL R2: 1 x 7 mL

Intended Use

The Diazyme Human Kappa (κ) 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 Hitachi 917 analyzers. The measurement of Kappa FLC in conjunction with Lambda FLC aids in the diagnosis of multiple myeloma in conjunction with other laboratory findings. For *in-vitro* diagnostic use only.

Clinical Significance

Normal immunoglobulins are composed of smaller units called heavy chains and light chains. There are five types of heavy chains (alpha, delta, epsilon, gamma, and mu), and two types of light chains (kappa and lambda). The heavy and light chains are produced separately within plasma cells and are assembled to form a whole ("intact") immunoglobulin. When the light chains are attached to the heavy chains they are called bound, and when they are not attached they are called free. Kappa Free Light Chain (FLC) is a 22kD protein while Lambda FLC is usually a dimer of 44kD as presented in the serum. Elevated levels of Kappa or Lambda FLC are associated with plasma cell disorders such as multiple myeloma, lymphocytic neoplasms, Waldenström's macroglobulinemia, AL amyloidosis, light chain deposition disease and connective tissue diseases such as systemic lupus erythematosus [1-5]

Assay Principle

Human Kappa (κ) Free Light Chain Assay is based on a latex enhanced immunoturbidimetric assay. Kappa FLC in the sample binds to specific anti-Kappa 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 in the sample. The instrument calculates the Kappa FLC concentration by interpolation of obtained signal of a 6-point calibration curve prepared from calibrators of known concentrations.

Reagent – "Working Solutions"

REAGENT 1: Tris Buffer Solution

REAGENT 2: Latex particles coated with polyclonal rabbit and goat anti-Kappa FLC antibodies

Reagent Handling

1. The Diazyme Human Kappa (κ) Free Light Chain Assay REAGENT provided is ready to use.
2. Deionized water is needed to dilute high Kappa FLC samples.

Reagent Stability and Storage

The Diazyme Human Kappa (κ) Free Light Chain Assay REAGENTS, CALIBRATORS (REF DZ169A-CAL) and CONTROLS (REF DZ169A-CON) should be stored at 2-8° C. **DO NOT FREEZE**. The REAGENTS, CALIBRATORS, and CONTROLS are stable when stored as instructed until the expiration date on the label. Do not mix REAGENTS from different lots.

Specimen Collection and Handling

Serum samples can be used for Human Kappa (κ) Free Light Chain Assay. Samples stored at 2-8°C are stable for 21 days; longer storage should be kept at -20°C. Repeated freeze/thaw cycles should be avoided to minimize potential protein degradation. Severely turbid samples should not be used.

Materials Provided

Please see the "Reagents – 'Working Solutions'" section.

Materials Required but not Provided

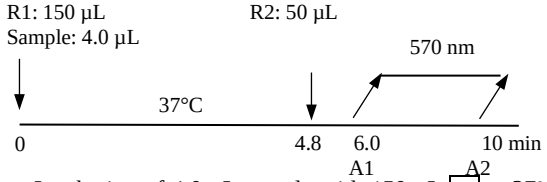
1. The Diazyme Human Kappa Lambda Free Light Chain CONTROLS Set (REF DZ169A-CON)
2. Deionized Water

Precautions

1. For *in-vitro* diagnostic use only.
2. Caution: Federal law restricts this device to sale by or on the order of a physician or other practitioner licensed by laws of the State in which he/she practices, to use or order the use of the device.
3. REAGENT, CALIBRATOR, and CONTROL should be stored at 2-8°C. DO NOT FREEZE.
4. Do not use the reagents, calibrator, and controls after the expiration date labeled on the outer box.
5. Specimens containing human sourced materials should be handled as if potentially infectious using safe laboratory procedures, such as those outlined in Biosafety in Microbiological and Biomedical Laboratories (HHS Publication Number [CDC] 93-8395).
6. Avoid ingestion and contact with skin and eyes. See the Safety Data Sheet (SDS).
7. The REAGENT contains <0.1% sodium azide, NaN_3, as preservative. Sodium azide may react with lead and copper to form highly explosive metal azide. On disposal, flush with a large volume of water to prevent azide buildup.
8. Additional safety information concerning storage or handling of this product is provided within the Safety Data Sheet (SDS) for this product. To obtain an SDS, please contact our customer service department at 858-455-4768.

Assay Procedures

Below is the assay procedure as run on the Hitachi 917 analyzer.



1. Incubation of 4.0 µL sample with 150 µL R1 at 37ºC for 4.8 minutes.

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2. Addition of 50 µL R2.
3. Reading of an absorbance change at 570nm for 4.0 minutes, 4 cycles after the addition of R2.
4. Calculation of Kappa FLC value with the read absorbance change by interpolation from a calibration curve prepared with calibrators of known concentrations.

For technical questions, please call 858-455-4768 or email: support@diazyme.com.

Calibration

Five levels of serum based calibrators (REF DZ169A-CAL) are available and ready for use. Use deionized H_2O and the provided calibrator levels 1-5 for calibration. The opened calibrators are stable for at least 1 month when stored at 2-8°C and capped tightly to minimize exposure to air and evaporation. Unopened calibrators are stable until expiration date printed on the vial.

Quality Control

We recommend that each laboratory use Diazyme Human Kappa/Lambda FLC controls to validate the performance of the Diazyme Human Kappa (κ) Free Light Chain Assay REAGENTS. A set of normal and abnormal ranges of Kappa FLC CONTROLS is available from Diazyme Laboratories (REF DZ169A-CON). The range of acceptable control limits should be established by individual laboratories. Follow federal, state and local guidelines for testing QC materials.

Results

Results are printed out in mg/L. Note: Samples with values greater than 150 mg/L should be diluted 1:20 with deionized water and reassayed. Multiply results by 20.

Reference Range

The reference interval of Diazyme Human Kappa (κ) Free Light Chain Assay was evaluated according to CLSI C28-A3 protocol with serum samples from 120 apparently healthy individuals. The central 95% reference interval of 2.37-20.73 mg/L was established.
The ratio of the Kappa (κ) FLC to Lambda (λ) FLC was evaluated. Serum samples from 315 apparently healthy individuals were tested with the Diazyme Kappa FLC Assay and Lambda FLC Assay. The ratio was calculated as Kappa FLC (A)/Lambda FLC (B). The total range of the ratio was from 0.22 to 1.74, mean ratio was 0.84, and median ratio was 0.81.
Each laboratory, however, is recommended to establish a range of normal values for the population in their region.

Limitations

1. There are no known international standards for Kappa FLC, however, this assay is predicated to a legally marketed device.
2. A sample with a Kappa FLC level exceeding 150 mg/L run in standard mode should be diluted with deionized water and re-assayed incorporating the dilution factor in the calculation of the value.
3. As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
4. The Diazyme Human Kappa (κ) Free Light Chain Assay has been validated on Hitachi 917, Abbott Architect c4000 /c8000, DZ-Lite c270, Roche cobas c501, Beckman AU680 and Ortho Clinical VITROS 4600/5600. Please contact technical support for updated application sheets at 858-455-4768 or email: support@diazyme.com

Performance Characteristics

The Diazyme Human Kappa (κ) Free Light Chain Assay performance was established on Hitachi 917 with a 6-point calibration using deionized water and separately provided calibrator levels 1-5. Results obtained from individual laboratories may vary.

Method Comparison

The method comparison of the Diazyme Human Kappa (κ) Free Light Chain Assay was evaluated following CLSI EP9-A2 protocol. A total of 126 serum samples ranging from 4.63-2975.80 mg/L were tested in comparison with predicate assay. Among the 126 samples, 39 were Multiple Myeloma (MM), 12 were Monoclonal Gammopathy of Undetermined Significance (MGUS), 34 were Abnormal (due to other disease states) and 41 were Normal (no disease), the concordance between the subject and predicate Kappa FLC is 98%. The results of regression analysis are summarized in the following table:

Parameter	Linear Regression	Deming Regression
n	126	126
Slope	0.958	0.969
95% CI	0.932-0.985	0.943-0.996
Intercept	-2.536	-4.813
95% CI	-14.703-9.632	-17.014-7.388
Corr Coeff (R^2)	0.977	0.977
Sample range	4.63-2975.80	4.63-2975.80

Precision

The precision of the Diazyme Human Kappa (κ) Free Light Chain Assay was evaluated according to CLSI EP5-A guideline. In the study, eight levels of serum specimens containing Kappa FLC spanning AMR and two levels of serum based Kappa FLC controls were tested with 2 runs per day with duplicates over 20 working days using multiple lots of the reagents on multiple analyzers. The precision data was analyzed according to three-way nested ANOVA and the results of mean (mg/L) and CV% are summarized below:
One lot of reagent on three analyzers

ID	Mean n=240	Within-Run	Between Run	Between day	Between Instr	Total
S1	9.38	7.6%	4.9%	3.5%	5.1%	11.0%
S2	35.30	2.1%	1.5%	1.3%	2.0%	3.5%
S3	122.37	2.4%	0.9%	1.8%	0.8%	3.2%
S4	5.95	7.5%	3.0%	8.5%	N/A	11.7%
S5	15.76	2.9%	1.6%	2.1%	0.2%	3.9%
S6	25.92	1.7%	1.3%	0.5%	0.4%	2.2%
S7	139.23	1.5%	1.5%	0.7%	1.1%	2.5%
S8	2588.59	1.4%	0.6%	2.6%	1.5%	3.3%
Con1	16.70	3.2%	2.6%	2.4%	0.9%	4.9%
Con2	29.10	2.1%	1.2%	2.1%	5.9%	6.7%

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Three lots of reagents on one analyzer

ID	Mean n=240	Within-Run	Be-tween Run	Be-tween day	Be-tween lot	Total
S1	9.49	7.9%	5.7%	5.7%	2.6%	11.6%
S2	35.14	2.9%	0.8%	1.5%	0.8%	3.5%
S3	121.53	2.6%	1.5%	2.3%	N/A	3.8%
S4	5.81	6.3%	2.6%	2.7%	0.7%	7.4%
S5	15.40	2.4%	0.9%	0.6%	2.2%	3.5%
S6	25.66	1.1%	0.3%	0.6%	1.0%	1.7%
S7	138.28	1.1%	N/A	1.0%	1.0%	1.8%
S8	2588.45	1.3%	1.0%	2.5%	1.5%	3.3%
Con1	16.64	4.2%	1.4%	3.0%	1.6%	5.6%
Con2	27.82	3.2%	N/A	2.7%	1.8%	4.6%

Detection Limits

The LOB, LOD, LOQ of the Diazyme Human Kappa (κ) Free Light Chain Assay was determined according to CLSI EP17-A2. The LOB was determined to be 1.2 mg/L; the LOD was determined to be 2.0 mg/L; the LOQ was determined to be 4.5 mg/L.

Linearity

The linearity of the Diazyme Human Kappa (κ) Free Light Chain Assay was evaluated according to CLSI EP6-A guideline. Based on the linearity data, limit of quantitation (LOQ = 4.5 mg/L), the Analytical Measuring Range (AMR) is claimed to be 4.5– 150 mg/L in Standard mode. The AMR is 4.5– 3000 mg/L including extended mode.

Interference

To determine the level of interference from the substances normally present in the serum, the Diazyme Human Kappa (κ) Free Light Chain Assay is tested with a normal Kappa FLC and an abnormal Kappa FLC (high) serum samples spiked with various concentrations of substances following CLSI EP7-A2 “Interference Testing in Clinical Chemistry” Approved guideline-Second Edition.

The following substances normally present in the serum produced less than 10% deviation when tested at levels equal to the concentrations listed below.

Interference	Concentration
Triglyceride	1000 mg/dL
Ascorbic Acid	10 mM
Bilirubin	40 mg/dL
Bilirubin Conjugated	40 mg/dL
Hemoglobin	1000 mg/dL
Rheumatoid Factor	100 IU/ml

Hook Effect

No high dose hook effect was observed up to 100,000 mg/L Kappa FLC.

References

1. International Myeloma Foundation «Understanding Serum Free Light Chain Assays», 2011
2. Drayson M, Tang LX, Drew R, Mead GP, Carr-Smith H and Bradwell AR. Serum free light-chain measurements for identifying and monitoring patients with nonsecretory multiple myeloma. Blood. 2001; 97(9):2900-2.
3. Nelson M, Brown RD, Gibson J and Joshua DE. Measurement of free kappa and lambda chains in serum and the significance of their ratio in patients with multiple myeloma. British Journal of Haematology. 1992; 81(2): 223-30.
4. Nakano T, Miyazaki S, Takahashi H, Matsumori A, Maruyama T, Komoda T and Nagata A. Immunochemical quantification of free immunoglobulin light chains from an analytical perspective. Clinical Chemistry Laboratory Medicine. 2006; 44(5): 522-32.
5. Bradwell AR, Carr-Smith HD, Mead GP, Harvey TC and Drayson MT. Serum test for assessment of patients with Bence Jones Myeloma. Lancet. 2003; 361: 489-91.

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