Human Immunoglobulin Free Light Chains Kappa and Lambda ELISA Kit

Cat. No.: DEIA4632
Pkg. Size: 96 T

**Intended use**

The Human Immunoglobulin Free Light Chains Kappa and Lambda ELISA kit contains two sandwich enzyme immunoassays for the quantitative measurement of human immunoglobulin free light chains kappa and lambda.

**General Description**

Human immunoglobulin molecules consist of two identical heavy chains which define immunoglobulin classes (IgG, IgA, IgM, IgD and IgE) and identical light chains (kappa or lambda) that are covalently linked to a heavy chain. In healthy individuals, the majority of light chains in serum exist bound to heavy chain. However, low levels of free light chains (FLCs) are found in serum of normal individuals due to their excess production over heavy chains by mature B-cells. In serum, FLC kappa exists predominantly as a monomer with a molecular weight of 22.5 kDa and FLC lambda as a dimer with a molecular weight of 45 kDa. This size difference results in a differential glomerular filtration rate and, consequently, a ratio of FLC kappa to FLC lambda of 1:1.6 in serum. FLCs are observed in urine too but filtration and reabsorption of low-molecular proteins in the kidney strongly affects the FLC concentration so that urinary FLC level is low in healthy individuals. FLCs are a natural product of B lymphocytes and, as such, represent a unique biomarker of neoplastic and reactive B cell-related disorders. Increased FLCs are associated with malignant plasma dyscrasias and other lymphocytorelated immunoproliferative disorders. The detection of the FLCs is important diagnostic aid for a variety of monoclonal gammopathies, such as multiple myeloma, Waldenstrom macroglobulinemia, nonsecretory myeloma, smoldering multiple myeloma, monoclonal gammapathy of undetermined significance. Accurate measurement of monoclonal free light chains in serum and/or urine is especially important in light-chain diseases, such as light-chain myeloma, primary systemic amyloidosis, and light-chain deposition disease. The ability to quantify monoclonal FLCs may be useful for monitoring the disease. In patients with light chain myeloma, either of light chain, kappa or lambda, is dominantly produced and resulting in marked changes of the FLC κ/λ ratio in the early phase of the disease. The detection of urinary monoclonal kappa or lambda free light chains of immunoglobulin, also known as Bence Jones proteins (BJP), are important for identifying and monitoring B cell malignancies too. In addition, compared with the healthy state, the synthesis of polyclonal FLCs markedly increased in conditions associated with B cell activation as found in certain inflammatory or autoimmune diseases (e.g. systemic lupus erythematosus, rheumatoid arthritis, or multiple sclerosis, as well as in cancer, diabetes mellitus, and AIDS).

**Principle Of The Test**

In the Human Immunoglobulin Free Light Chains Kappa and Lambda ELISA, standards, quality controls and samples are incubated in microplate wells pre-coated with monoclonal anti-human immunoglobulin FLC kappa or FLC lambda antibody. After 60 minutes incubation and washing, biotin-labelled second monoclonal antibody is added and incubated with captured antibody-FLC kappa or lambda complex for 60 minutes. After another washing, streptavidin-HRP conjugate is added. After 30 minutes incubation and the last washing step, the remaining conjugate is allowed to react with the substrate solution (TMB). The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured. The absorbance is proportional to the concentration of FLC. A standard curve is constructed by plotting absorbance values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

**Reagents And Materials Provided**

1. Human FLC kappa Antibody Coated Microtiter Strips, ready to use, 96 wells
2. Human FLC kappa Biotin Labelled Antibody Conc. (30x), concentrated, 0.45 ml
3. Human FLC kappa Master Standard, lyophilized, 2 vials
4. Human FLC kappa Quality Control HIGH, lyophilized, 2 vials
5. Human FLC kappa Quality Control LOW, lyophilized, 2 vials
6. Human FLC lambda Antibody Coated Microtiter Strips, ready to use, 96 wells
7. Human FLC lambda Biotin Labelled Antibody Conc. (30x), concentrated, 0.45 ml
8. Human FLC lambda Master Standard, lyophilized, 2 vials
9. Human FLC lambda Quality Control HIGH, lyophilized, 2 vials
10. Human FLC lambda Quality Control LOW, lyophilized, 2 vials
11. Streptavidin-HRP Conjugate Conc. (30x), concentrated, 1.0 ml
12. Dilution Buffer Conc. (10x), concentrated, 22 ml
13. Wash Solution Conc. (10x), concentrated, 2 x 100 ml
14. Substrate Solution, ready to use, 2 x 13 ml
15. Stop Solution, ready to use, 2 x 13 ml
16. Product Data Sheet - 1 pc

**Materials Required But Not Supplied**

1. Deionized (distilled) water
2. Test tubes for diluting samples
3. Glassware (graduated cylinder and bottle) for Wash Solution (Dilution Buffer)
4. Precision pipettes to deliver 5-1000μl with disposable tips
5. Multichannel pipette to deliver 100 μl with disposable tips
6. Absorbent material (e.g. paper towels) for blotting the microtitrate plate after washing
7. Vortex mixer
8. Orbital microplate shaker capable of approximately 300 rpm
10. Microplate reader with 450 ± 10 nm filter, preferably with reference wavelength 630 nm (alternatively another one from the interval 550-650 nm)
11. Software package facilitating data generation and analysis (optional)

**Storage**

Store the complete kit at 2-8°C. Under these conditions, the kit is stable until the expiration date (see label on the box).

**Specimen Collection And Handling**

The kit measures FLC kappa and FLC lambda in serum, plasma (EDTA, citrate, heparin) and urine. Samples should be assayed immediately after collection or should be stored at -20°C. Mix thoroughly thawed samples just prior to the assay and avoid repeated freeze/thaw cycles, which may cause erroneous results. Avoid using hemolyzed or lipemic samples.

Dilution factor of samples is the same for both ELISAs and therefore dilute samples in enough amounts for both assays.

**Serum or plasma samples:**

Dilute samples 200x with Dilution Buffer just prior to the assay, e.g. 5μl of sample + 995μl Dilution Buffer for duplicates or singlets. Mix well (not to foam). Vortex is recommended.

Results exceeding FLC kappa level of 320μg/l or FLC lambda level of 560μg/l should by repeated with more diluted samples. It is recommended to dilute samples just prior to assay 2 000-fold and 20 000-fold in next assay as follows (for duplicates and singlets):

50 μl of dilution 200-fold + 450 μl of Dilution Buffer for final dilution 2 000-fold 5 μl of dilution 200-fold + 495μl of Dilution Buffer for final dilution 20 000-fold
Dilution factor needs to be taken into consideration in calculating of the FLC kappa or FLC lambda concentration.

Urine samples:
Dilute urine samples from healthy 40x with Dilution Buffer just prior to the assay, e.g. 15 μl of sample + 585μl of Dilution Buffer, and for patient 200x, e.g. 5 μl of sample + 995μl of Dilution Buffer. Mix well (not to foam). Vortex is recommended.

Stability and storage:
Serum and plasma samples should be stored at -20°C, or preferably at -70°C for long-term storage. Urine samples should be stored at -70°C.
Do not store the diluted samples.

Note: It is recommended to use a precision pipette and a careful technique to perform the dilution in order to get precise results.

Reagent Preparation

All reagents need to be brought to room temperature prior to use Always prepare only the appropriate quantity of reagents for your test Do not use components after the expiration date marked on their label.

Assay reagents supplied ready to use:
Antibody Coated Microtiter Strips (FLC kappa or lambda)

Stability and storage:
Return the unused strips to the provided aluminium zip-sealed bag with desiccant and seal carefully. Remaining Microtiter Strips are stable 3 months when stored at 2-8°C and protected from the moisture.

Substrate Solution
Stop Solution

Stability and storage:
Opened reagents are stable 3 months when stored at 2-8°C.

Assay reagents supplied concentrated or lyophilized:

Dilution Buffer Conc. (10x)

Dilute Dilution Buffer Concentrate (10x) ten-fold in distilled water to prepare a 1x working solution. Example: 20 ml of Dilution Buffer Concentrate (10x) + 180 ml of distilled water for use of all 96-wells.

Dilution Buffer is same for dilution of FLC Kappa and Lambda Standards, Quality Controls, samples, Biotin Labelled Antibody Concentrates and Streptavidin-HRP Conjugate Concentrate.

Stability and storage:
The diluted Dilution Buffer is stable 1 month when stored at 2-8°C. Opened Dilution Buffer Concentrate (10x) is stable 3 months when stored at 2-8°C.

FLC kappa Master Standard

Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution of standard!!!
Reconstitute the lyophilized Master Standard with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam). The resulting concentration of the FLC kappa in the stock solution is 320 μg/l.
Prepared Standards are ready to use, do not dilute them.

Stability and storage:
Do not store the Standard stock solution and set of standards.

FLC kappa Quality Controls HIGH, LOW

Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution and for current Quality Control concentration!!!
Reconstitute each Quality Control (HIGH and LOW) with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam).
Reconstituted Quality Controls are ready to use, do not dilute them.

Stability and storage:
Do not store the reconstituted Quality Controls.
Note:
Concentration of analyte in Quality Control need not be anyhow associated with normal and/or pathological concentrations in serum or another body fluid. Quality Control serves just for control that the kit works in accordance with PDS and CoA and that ELISA test was carried out properly.

FLC kappa Biotin Labelled Antibody Conc. (30x)
Prepare the working Biotin Labelled Antibody solution by adding 1 part Biotin Labelled Antibody Concentrate (30x) with 29 parts Dilution Buffer.
Example: 33 μl of Biotin Labelled Antibody Concentrate (30x) + 957μl of Dilution Buffer for 1 strip (8 wells).

Stability and storage:
Opened Biotin Labelled Antibody Concentrate (30x) is stable 3 months when stored at 2-8°C.
Do not store the diluted Biotin Labelled Antibody solution.

FLC lambda Master Standard
Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution of standard!!!
Reconstitute the lyophilized Master Standard with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam). The resulting concentration of the FLC lambda in the stock solution is 560 μg/l.
Prepared Standards are ready to use, do not dilute them.

Stability and storage:
Do not store the Standard stock solution and set of standards.

FLC lambda Quality Controls HIGH, LOW
Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution and for current Quality Control concentration!!!
Reconstitute each Quality Control (HIGH and LOW) with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam).
Reconstituted Quality Controls are ready to use, do not dilute them.

Stability and storage:
Do not store the reconstituted Quality Controls.

Note:
Concentration of analyte in Quality Control need not be anyhow associated with normal and/or pathological concentrations in serum or another body fluid. Quality Control serves just for control that the kit works in accordance with PDS and CoA and that ELISA test was carried out properly.

FLC lambda Biotin Labelled Antibody Conc. (30x)
Prepare the working Biotin Labelled Antibody solution by adding 1 part Biotin Labelled Antibody Concentrate (30x) with 29 parts Dilution Buffer.
Example: 33 μl of Biotin Labelled Antibody Concentrate (30x) + 957μl of Dilution Buffer for 1 strip (8 wells).

Stability and storage:
Opened Biotin Labelled Antibody Concentrate (30x) is stable 3 months when stored at 2-8°C.
Do not store the diluted Biotin Labelled Antibody solution.

Streptavidin-HRP Conjugate Conc. (30x)
Prepare the working Streptavidin-HRP Conjugate solution by adding 1 part Streptavidin-HRP Conjugate Concentrate (30x) with 29 parts Dilution Buffer. Example: 33 μl of Streptavidin-HRP Conjugate Concentrate (30x) + 957μl of Dilution Buffer for 1 strip (8 wells).

Streptavidin-HRP Conjugate solution is same for both ELISAs.
Stability and storage:
Opened Streptavidin-HRP Conjugate Concentrate (30x) is stable 3 months when stored at 2-8°C.
Do not store the diluted Streptavidin-HRP Conjugate solution.

Wash Solution Conc. (10x)
Dilute Wash Solution Concentrate (10x) ten-fold in distilled water to prepare a 1x working solution. Example: 100 ml of Wash Solution Concentrate (10x) + 900 ml of distilled water for use of all 96-wells.

Stability and storage:
The diluted Wash Solution is stable 1 month when stored at 2-8°C. Opened Wash Solution Concentrate (10x) is stable 3 months when stored at 2-8°C.

**Assay Steps**

1. Pipet 100μl of diluted Standards, Quality Controls, Dilution Buffer (=Blank) and samples, preferably in duplicates, into the appropriate wells.
2. Incubate the plate at room temperature (ca. 25°C) for 1 hour, shaking at ca. 300 rpm on an orbital microplate shaker.
3. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
4. Add 100 μl of Biotin Labelled Antibody solution into each well.
5. Incubate the plate at room temperature (ca. 25°C) for 1 hour, shaking at ca. 300 rpm on an orbital microplate shaker.
6. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
7. Add 100μl of Streptavidin-HRP Conjugate solution into each well.
8. Incubate the plate at room temperature (ca. 25°C) for 30 min, shaking at ca. 300 rpm on an orbital microplate shaker.
9. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
10. Add 100μl of Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.
11. Incubate the plate for 15 minutes at room temperature. The incubation time may be extended [up to 25 minutes] if the reaction temperature is below than 20°C. Do not shake the plate during the incubation.
12. Stop the colour development by adding 100 μl of Stop Solution.
13. Determine the absorbance of each well using a microplate reader set to 450 nm, preferably with the reference wavelength set to 630 nm (acceptable range: 550 - 650 nm). Subtract readings at 630 nm (550 - 650 nm) from the readings at 450 nm.

The absorbance should be read within 5 minutes following step 12.

Note: If some samples and standard/s have absorbances above the upper limit of your microplate reader, perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine FLC concentration of off-scale standards and samples. The readings at 405 nm should not replace the readings for samples that were “in range” at 450 nm.

Note 2: Manual washing: Aspirate wells and pipet 0.35 ml Wash Solution into each well. Aspirate wells and repeat twice. After final wash, invert and tap the plate strongly against paper towel. Make certain that Wash Solution has been removed entirely.

**Calculation**

Most microplate readers perform automatic calculations of analyte concentration. The standard curve is constructed by plotting the mean absorbance (Y) of Standards against the known concentration (X) of Standards in logarithmic scale, using the four-parameter algorithm. Results are reported as concentration of FLC g/l in samples.

Alternatively, the logit log function can be used to linearize the standard curve, i.e. logit of the mean absorbance (Y) is plotted against log of the known concentration (X) of Standards.

The measured concentration of samples calculated from the standard curve must be multiplied by their respective dilution factor, because samples have been diluted prior to the assay, e.g. 100 μg/l (from standard curve) x 200 (dilution factor) = 20 000 μg/l = 20 mg/l.
Typical Standard Curve

This standard curve is provided for demonstration only. A standard curve should be generated for each assay plate. Typical sIL-4R standard curve ranging from 31.25 to 1000 pg/mL.
Reference Values

The following results were obtained when serum samples from 40 unselected donors 41-64 years old were assayed with the Human Immunoglobulin Free Light Chains Kappa and Lambda ELISA in external laboratory.

- **Normal value**
  - FLC kappa normal value (mean +/- SEM) is 11.5 +/- 0.58 mg/l
  - FLC kappa normal range (mean +/- 2xSD) is 11.5 +/- 6.72 mg/l
  - FLC lambda normal value (mean +/- SEM) is 17.39 +/- 1.08 mg/l
  - FLC lambda normal range (mean +/- 2xSD) is 17.39 +/- 13.68 mg/l
  - FLC kappa/lambda ratio normal value (mean +/- SEM) is 0.707 +/- 0.037
  - FLC kappa/lambda ratio normal range (mean +/- 2xSD) is 0.707 +/- 0.466

- **Reference range**
  

  The data quoted in these instructions should be used for guidance only. It is recommended that each laboratory include its own panel of control sample in the assay. Each laboratory should establish its own normal and pathological references ranges for FLCs levels with the assay. The following results were obtained when serum from 160 unselected blood donors (80 Women + 80 Men) 5-86 years old were assayed with Human Immunoglobulin Free Light Chain Kappa and Lambda ELISA kit in our laboratory.

Sensitivity

Limit of Detection (LOD) (defined as concentration of analyte giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: Ablank + 3xSDblank) is calculated from the real FLC kappa values in wells and is 6μg/l.

* Dilution Buffer is pipetted into blank wells.
Specificity

The antibodies used in this ELISA are specific for human FLC kappa. Sera of several mammalian species were measured in the assay.

Linearity

Serum samples were serially diluted with Dilution Buffer and assayed.
Linearity: 83.7 - 90.5 %

Recovery

Serum samples were spiked with different amounts of FLC kappa and assayed.
Recovery: 95.9 - 104.9 %

Reproducibility

Intra-Assay(n =8): 2.0 - 4.0%
Inter-Assay(n =8): 6.9 - 7.0%

Precautions

1. Wear gloves and laboratory coats when handling immunodiagnostic materials
2. Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled
3. This kit contains components of human origin. These materials were found non-reactive for HBsAg, HCV antibody and for HIV 1/2 antigen and antibody. However, these materials should be handled as potentially infectious, as no test can guarantee the complete absence of infectious agents
4. Avoid contact with the acidic Stop Solution and Substrate Solution, which contains hydrogen peroxide and tetramethylbenzidine (TMB). Wear gloves and eye and clothing protection when handling these reagents. Stop and/or Substrate Solutions may cause skin/eyes irritation. In case of contact with the Stop Solution and the Substrate Solution wash skin/eyes thoroughly with water and seek medical attention, when necessary
5. The materials must not be pipetted by mouth

Limitations

Results exceeding FLC kappa level of 320 μg/l should be repeated with more diluted samples. Dilution factor needs to be taken into consideration in calculating the FLC kappa concentration.

REFERENCES