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CE IVD

EN

INSTRUCTIONS FOR USE

Reagents for professional use,
for *In Vitro* use only in clinical laboratory (IVD)

κλoneus® - U-FLC-K - TIA

Free Light Chains KAPPA - Urine
for *Turbidimetry*

REF TD-42511-UK

(Product included in REF TD-42510-U)

INTENDED USE

Quantitative determination of Free Light Chains KAPPA (FLC-K) in human urine, by turbidimetric method, in automatic Clinical Chemistry Analyzers.

PRINCIPLE OF THE METHOD

The specific antibodies (Ab) of the reagent, bound to polystyrene particles, when combined with the antigens (Ag) of the patient sample, form insoluble compounds causing a change in the absorbance and dispersion of the light, proportional to the antigen concentration, which can be quantified by turbidimetric (TIA) or nephelometric (NIA) method, by comparison with calibrators of known concentration.

CONTENTS - COMPOSITION - PREPARATION

- Antiserum Reagent: REAG | Ab | U-FLC-K
  REF TD-42511-RUK 100 test (*1) - 5 ml
  Polyclonal antibodies bound to polystyrene particles.
- Reaction Buffer: BUF | U-FLC-K
  REF TD-42511-BUK 100 test (*1) - 20 ml
  TRIS Buffer, containing PEG.

Note (*1): with the recommended general assay parameters.

The reagents are ready for use and require no preparation.

Before each use it is convenient that the reagents are homogenized, shaking them gently avoiding the formation of foam or bubbles.

As preservative, the reagents contain <0.1% (1 g/l) Sodium Azide (NaN_3).

WARNINGS - PRECAUTIONS

- Sodium Azide is toxic. Even if sodium azide is not harmful at the concentration present in the reagents, take the necessary precautions to avoid accidental ingestion or contact with the eyes.
- Sodium Azide can react with lead or copper to give explosive compounds. For disposal it is recommended to rinse with plenty of running water to avoid accumulation in drains.
- Materials of human origin have been tested and found negative for the presence of HBsAg, HCV, and anti-HIV 1 and 2 antibodies.
- Since the absence of infectious agents can not be proven with absolute certainty, components containing materials of human or animal origin must be handled with caution, as potentially infectious, following the recommended safety standards for biological risk.
- Do not mix components belonging to different lot kits.
- Clinical diagnosis should not be based on the results of a single test, but should always integrate all relevant clinical and laboratory data.

STORAGE - SHELF LIFE

- Store refrigerated at +2...+8ºC. Do not freeze, as the functionality of the reagents may be altered.
- Properly stored and unopened, the reagents are stable until the expiration date indicated on the label.
- Once opened, the shelf life of the reagents is at least 4 weeks, provided that after each use they are stored immediately in the original containers, tightly capped and refrigerated at +2...+8ºC. This information should be taken as a guideline given that, obviously, the shelf life depends on the particular environmental and use conditions, which may differ from those of the stability studies carried out.

MATERIALS NEEDED, NOT SUPPLIED

- Automatic Clinical Chemistry Analyzer, capable of running photometric assays at 600...700 nm, and accessories: reagent containers, cuvettes, etc..
- κλoneus® - U-FLC - Cal Set REF TD-42501-U
- κλoneus® - U-FLC - Control REF TD-42502-U

SAMPLES

Fresh urine.

It is usual the use of a 24-hour urine aliquot, however specific guidelines[1] recommend the use of a random urine, preferably the second morning void, and expressing the results relative to urinary creatinine. The addition of <0.1% (1 g/l) Sodium Azide (NaN_3) as a preservative is also recommended.

Prior to the analysis, the samples should be centrifuged until a clear and transparent supernatant is obtained[2].

For the determination of specific proteins, centrifugation of urine samples at 3000[3]-5000[4] g for 10 minutes is the standard practice in the laboratory.

In bibliography[5] it is reported a stability of 7 days in refrigerated urine (sample of preference). The sample should always be kept refrigerated.

PROCEDURE

If necessary, carefully transfer the reagents to the containers used by the analyzer, preventing leakage and foaming or bubbles.

To program and calibrate assays, follow the instructions for use of the analyzer used, with the recommended general parameters that are detailed below. Please, contact the Customer Support Service (support@3diag.com - ☎ +34 93 244 86 79) for further information about applications to specific analyzers.

Assay Parameters

- ①Dispense and mix:
  - Sample/Calibrator/Control: 5 µl (dilute 1:5)
  - BUF | U-FLC-K 200 ul
- ②Incubate a fixed time between 1 and 5 minutes
- ③Dispense and mix:
  - REAG | Ab | U-FLC-K 50 ul
- ④Read absorbance A1 (Blank) at 600...700 nm
- ⑤Incubate a fixed time of about 5 minutes
- ⑥Read absorbance A2 (Final Point) at 600...700 nm
- ⑦Interpolate the absorbance increment (A2-A1) of the samples and controls in the curve obtained with the calibrators
- ⑧Samples with concentrations higher than the upper limit of the assay range should be analyzed again, manually diluted with Physiological Solution, or by programming a larger sample dilution in the analyzer, to recover a value close to the midpoint of the measurement range.

As an alternative, reagents can be mixed as first step, and the sample dispensed as starter.

Calibration Parameters

- Calibrators: Use the κλoneus® - U-FLC - Cal Set.
- If the analyzer allows it, it is recommended to program two replicates of each calibration point.

- The calibration is Non-linear. For the calculation it is recommended to use a 3rd Order Polynomial, Logit or Polygonal adjustment.

The assay must be recalibrated, at least when a new batch of reagents is used or when its parameterization is changed.

PERFORMANCES OF THE METHOD

Detailed information on the characteristics and performances of the assays is given in the Technical Reports, available on the website (www.3diag.com) or upon request to the Customer Support Service (support@3diag.com - ☎ +34 93 244 86 79).

Antigen Excess

The Free Light Chains (FLC) of the sample, especially if they are monoclonal, can react in a way that is not proportional to the calibration (lack of linearity), just as it happens in the immunochemical quantification of monoclonal immunoglobulins.

Although the method does not enter into antigen excess until very high concentrations of FLC, as a precaution it is recommended to analyze patient samples, which, because of their history, clinical data or other laboratory results, are suspected of having FLC extreme values or whose reaction is non-proportional, at two dilutions, the usual working one and manually prediluted (for example 1:10). Recovered result of the prediluted sample significantly higher than that of the sample at the normal dilution is indicative of an eventual excess of antigen or non-linearity; in that case, to obtain a result as accurate as possible, it is recommended to dilute the sample progressively (for example in steps of 1:5) until a value close to the midpoint of the measurement range is recovered.

The use of complementary assays, for example the determination in urine of the Total Light Chains (free+bound) together with the FLC, the determination of the FLC at the same time in serum and urine, or electrophoretic assays, can be a useful alarm signal in case of obtaining discordant results.

QUALITY CONTROL

To monitor performances, it is recommended that internal controls be inserted into each analytical series. It is recommended to use the controls of κλoneus® - U-FLC - CONTROL.

Each laboratory should establish its own quality scheme and corrective actions if controls do not meet the assigned tolerances.

The reagents have been subjected to quality control checks and should react as described in these instructions. Therefore, as a general recommendation, in case the controls do not give the expected reaction, as a precaution all reagents should be considered unreliable until their operation has been checked.

TRACEABILITY

Given that certified reference materials are not available, in order to ensure traceability, values have been assigned based on the measurement of the Light Chains in the calibrators (pure solutions of FLC-K and FLC-L), with a nephelometric method standardized to the *European Reference Material ERM-DA470k/IFCC* (*Institute for Reference Materials and Measurements*, *IRMM*), using the formula of *M.M. Lievens* [6].

Values are also referred to internal standards based on highly purified proteins.

REFERENCE INTERVALS

It is always advisable for each laboratory to establish its own reference values.

In general, FLC are present only in traces in the urine of normal subjects. Specific guidelines[1] report that, for the study of monoclonal components, at least 1 mg/dl (10 mg/l) of Kappa and Lambda FLC should be detected, concentration which has therefore be considered as significant.

CLINICAL SIGNIFICANCE

Immunoglobulin molecules are composed of two identical heavy chains (HC) of the same type and two identical light chains (LC) of the same type, linked by a variable number of disulphide bridges and non-covalent links. The amount of LC and HC produced by plasma cells is unbalanced, resulting in an excess of LC (FLC = Free Light Chains) that are secreted in the serum and, given their low molecular weight (approx. 22-25 KDa for the monomers), are almost completely eliminated by the kidney.

In the so-called monoclonal gammopathies, plasma cells frequently generate large (sometimes huge) quantities of FLC, which have the particular characteristic of being monoclonal (i.e. produced by a single clone). This hyperproduction of monoclonal FLC causes, in addition to the increase of its concentration in the serum, to overcome the tubular reabsorption capacity in the kidney and then FLC are also found in the urine, which is normally known as Bence Jones Proteinuria (BJP). The amount of FLC in serum is determined by the balance between their production and their renal clearance (glomerular filtration), which depends on their degree of polymerization. The amount in urine will also depend on their tubular reabsorption rate.

Quantities of FLC, both in serum and in urine, exceeding normal values or an abnormal κ/λ FLC ratio may be indicative of the presence of a monoclonal gammopathy, which should always be confirmed by electrophoretic techniques. Its quantification may also be useful in monitoring the monoclonal component.

In urine, specific guidelines[1] propose, as an alternative approach, the use of the quantitative measurement of FLC as a screening method for the presence of Bence-Jones proteinuria (BJP), that may also be useful in monitoring and as BJP quantitative estimation, more precise and sensitive than the one made electrophoretically.

SYMBOLS

In addition to the harmonized symbols provided on the European Standard EN 980:2008, in the labels and instructions of use has been used the complementary symbology proposed[7] by the *EDMA* (*European Diagnostic Manufacturers Association*), whose meaning is detailed below.

REAG	Reagent
Ab	Antibody / Antiserum
U-FLC-K	Free Light Chains KAPPA - Urine
BUF	Buffer
CONT	Contents

BIBLIOGRAPHY

(1) Graziani et al. for the *IFCC Committee on Plasma Proteins*: "*Guidelines for the Analysis of Bence Jones Protein*" - Clin Chem Lab Med 2003; 41(3): 338-346.

(2) Morales LJ., Ventura S., Solé E et al. - Comite de Comunicación de la Sociedad Española de Medicina de Laboratorio, SEQC[ML]: "*Muestras de Orina de 24 horas y Orina Reciente para la Medición de las Magnitudes Biológicas Más Comunes*", ISBN: 978-84-89975-52-1 (2017).

(3) "*Alpha-1-Microglobulin (A1M) - IMMAGE® Immunochemistry Systems Chemistry Information Sheet*", © Copyright 2017 Beckman Coulter, Inc..

(4) Bergón Jiménez E., Bergón Sendín M.: "*Uso del cociente cadenas kappa/cadenas lambda en orina para el estudio de la proteína de Bence Jones*", Química Clínica 1999; 18 (5) 266-270.

(5) *Mayo Medical Laboratories website* (www.mayomedicallaboratories.com), date of consultation: 7th September 2017.

(6) M.M. Lievens: "*Medical and technical usefulness of measurement of kappa and lambda immunoglobulin light chains in serum with an M-component*" - J Clin Chem Clin Biochem 1989; 27; 519-23.

(7) EDMA Labelling Task Force: "*EDMA Symbols for IVD Reagents and Components - Revision, October 2009*".

TEXT REVISION DATE

16th July 2020.

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INSTRUCTIONS FOR USE

Reagents for professional use,
for *In Vitro* use only in clinical laboratory (IVD)

κλoneus® - U-FLC - CAL SET

Free Light Chains - Urine
Calibrators (6 lev.)
REF **TD-42501-U**
(Product included in REF TD-42510-U)

INTENDED USE

Elaboration of the calibration curve for the quantitative determination of Free Light Chains Kappa and Lambda (FLC-K and FLC-L) in human urine, by immunochemical methods, with κλoneus® reagents

PRINCIPLE OF THE METHOD

The specific antibodies (Ab) of the reagent, bound to polystyrene particles, when combined with the antigens (Ag) of the patient sample, form insoluble compounds causing a change in the absorbance and dispersion of the light, proportional to the antigen concentration, which can be quantified by turbidimetric (TIA) or nephelometric (NIA) method, by comparison with calibrators of known concentration.

CONTENTS - COMPOSITION - PREPARATION

• Level 1:	CAL 1 U-FLC	REF	TD-42501-U1	CONT	1 ml
• Level 2:	CAL 2 U-FLC	REF	TD-42501-U2	CONT	1 ml
• Level 3:	CAL 3 U-FLC	REF	TD-42501-U3	CONT	1 ml
• Level 4:	CAL 4 U-FLC	REF	TD-42501-U4	CONT	1 ml
• Level 5:	CAL 5 U-FLC	REF	TD-42501-U5	CONT	1 ml
• Level 6:	CAL 6 U-FLC	REF	TD-42501-U6	CONT	1 ml

The calibrators are human Free Light Chain solutions.
As preservatives, the calibrators contain <0.1% (1 g/l) Sodium Azide (NaN_3), <0.02% (0.2 g/l) Methylisothiazolone and <0.02% (0.2 g/l) Bromonitrodioxane.
The calibrators are ready for use and require no preparation.
Before each use it is convenient that the calibrators are homogenized, shaking them gently avoiding the formation of foam or bubbles.
It is always advisable to bring the calibrators to room temperature before use.
The values of the calibrators are lot dependent and are indicated in the table of values of their Instructions for Use.

WARNINGS - PRECAUTIONS

- Sodium Azide is toxic. Even if at the concentrations present neither Sodium Azide nor the other preservatives are harmful, take the necessary precautions to avoid accidental ingestion or contact with the eyes.
- Sodium Azide can react with lead or copper to give explosive compounds. For disposal it is recommended to rinse with plenty of running water to avoid accumulation in drains.
- Materials of human origin have been tested and found negative for the presence of HBsAg, HCV, and anti-HIV 1 and 2 antibodies.
- Since the absence of infectious agents can not be proven with absolute certainty, components containing materials of human or animal origin must be handled with caution, as potentially infectious, following the recommended safety standards for biological risk.
- Do not mix components belonging to different lot kits.
- Clinical diagnosis should not be based on the results of a single test, but should always integrate all relevant clinical and laboratory data.

STORAGE - SHELF LIFE

- Store refrigerated at +2...+8ºC. Do not freeze, as the functionality of the calibrators may be altered.
- Properly stored and unopened, the calibrators are stable until the expiration date indicated on the label.
- Once opened, the shelf life of the calibrators is at least 4 weeks, provided that after each use they are stored immediately in the original containers, tightly capped and refrigerated at +2...+8ºC. This information should be taken as a guideline given that, obviously, the shelf life depends on the particular environmental and use conditions, which may differ from those of the stability studies carried out.

MATERIALS NEEDED, NOT SUPPLIED

The calibrators are intended to be used in conjunction with the Reagents and Controls (available separately or included in Kits):

• κλoneus® - U-FLC - 800	REF TD-42500-RU
• κλoneus® - U-FLC-K - TIA	REF TD-42511-UK
• κλoneus® - U-FLC-L - TIA	REF TD-42511-UL
• κλoneus® - U-FLC - CONTROL	REF TD-42502-U

PROCEDURE

Follow the Instructions for Use of the analyzer used to program and calibrate an assay, with the general parameters recommended in the Instructions for Use of Reagents.

TRACEABILITY

Given that certified reference materials are not available, in order to ensure traceability, values have been assigned based on the measurement of the Light Chains in the calibrators (pure solutions of FLC-K and FLC-L), with a nephelometric method standardized to the *European Reference Material ERM-DA470k/IFCC* (*Institute for Reference Materials and Measurements*, *IRMM*), using the formula of *M.M. Lievens*[(1)].
Values are also referred to internal standards based on highly purified proteins.

SYMBOLS

In addition to the harmonized symbols provided on the European Standard EN 980:2008, in the labels and instructions of use has been used the complementary symbology proposed[(2)] by the *EDMA* (*European Diagnostic Manufacturers Association*), whose meaning is detailed below.

CAL	Calibrator
n	Level n (n=1..6)
U-FLC	Free Light Chains - Urine
CONT	Contents

BIBLIOGRAPHY

(1) M.M. Lievens: "*Medical and technical usefulness of measurement of kappa and lambda immunoglobulin light chains in serum with an M-component*" - J Clin Chem Clin Biochem 1989; 27; 519-23.
(2) EDMA Labelling Task Force: "*EDMA Symbols for IVD Reagents and Components - Revision, October 2009*".

TEXT REVISION DATE

2nd July 2020.

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INSTRUCTIONS FOR USE

Reagents for professional use,
for *In Vitro* use only in clinical laboratory (IVD)

κλoneus® - U-FLC - CONTROL

Free Light Chains - Urine
Controls (3 lev.)
REF **TD-42502-U**
(Product included in REF TD-42510-U)

INTENDED USE

Internal controls, with assigned values, for the quantitative determination of Free Light Chains Kappa and Lambda (FLC-K and FLC-L) in human urine, by immunochemical methods, with κλoneus® reagents

PRINCIPLE OF THE METHOD

The specific antibodies (Ab) of the reagent, bound to polystyrene particles, when combined with the antigens (Ag) of the patient sample, form insoluble compounds causing a change in the absorbance and dispersion of the light, proportional to the antigen concentration, which can be quantified by turbidimetric (TIA) or nephelometric (NIA) method, by comparison with calibrators of known concentration.

CONTENTS - COMPOSITION - PREPARATION

- High Control: CONTROL | H | U-FLC
  REF TD-42502-UH CONT 1 ml
- Medium Control: CONTROL | M | U-FLC
  REF TD-42502-UM CONT 1 ml
- Low Control: CONTROL | L | U-FLC
  REF TD-42502-UL CONT 1 ml

The controls are human Free Light Chain solutions.
As preservatives, the controls contain <0.1% (1 g/l) Sodium Azide (NaN_3), <0.02% (0.2 g/l) Methylisothiazolone and <0.02% (0.2 g/l) Bromonitrodioxane.
The controls are ready for use and require no preparation.
Before each use it is convenient that the controls are homogenized, shaking them gently avoiding the formation of foam or bubbles.
The values of the controls are lot dependent and are indicated in the table of values of their Instructions for Use.

WARNINGS - PRECAUTIONS

- Sodium Azide is toxic. Even if at the concentrations present neither Sodium Azide nor the other preservatives are harmful, take the necessary precautions to avoid accidental ingestion or contact with the eyes.
- Sodium Azide can react with lead or copper to give explosive compounds. For disposal it is recommended to rinse with plenty of running water to avoid accumulation in drains.
- Materials of human origin have been tested and found negative for the presence of HBsAg, HCV, and anti-HIV 1 and 2 antibodies.
- Since the absence of infectious agents can not be proven with absolute certainty, components containing materials of human or animal origin must be handled with caution, as potentially infectious, following the recommended safety standards for biological risk.
- Do not mix components belonging to different lot kits.
- Clinical diagnosis should not be based on the results of a single test, but should always integrate all relevant clinical and laboratory data.

STORAGE - SHELF LIFE

- Store refrigerated at +2...+8ºC. Do not freeze, as the functionality of the controls may be altered.
- Properly stored and unopened, the controls are stable until the expiration date indicated on the label.
- Once opened, the shelf life of the controls is at least 4 weeks, provided that after each use they are stored immediately in the original containers, tightly capped and refrigerated at +2...+8ºC. This information should be taken as a guideline given that, obviously, the shelf life depends on the particular environmental and use conditions, which may differ from those of the stability studies carried out.

MATERIALS NEEDED, NOT SUPPLIED

The controls are intended to be used in conjunction with the Reagents and Calibrators (available separately or included in the Kits):

- κλoneus® - U-FLC-K - TIA — REF TD-42511-UK
- κλoneus® - U-FLC-L - TIA — REF TD-42511-UL
- κλoneus® - U-FLC - CAL SET — REF TD-42501-U

PROCEDURE

Follow the Instructions for Use of the analyzer used to program and calibrate an assay, with the general parameters recommended in the Instructions for Use of Reagents.

TRACEABILITY

Given that certified reference materials are not available, in order to ensure traceability, values have been assigned based on the measurement of the Light Chains in the calibrators (pure solutions of FLC-K and FLC-L), with a nephelometric method standardized to the *European Reference Material ERM-DA470k/IFCC* (*Institute for Reference Materials and Measurements*, *IRMM*), using the formula of *M.M. Lievens*[1].
Values are also referred to internal standards based on highly purified proteins.

SYMBOLS

In addition to the harmonized symbols provided on the European Standard EN 980:2008, in the labels and instructions of use has been used the complementary symbology proposed[2] by the *EDMA* (*European Diagnostic Manufacturers Association*), whose meaning is detailed below.

CONTROL	Control
X	Level X (H=High, M=Medium y L=Low)
U-FLC	Free Light Chains - Urine
CONT	Contents

BIBLIOGRAPHY

(1) M.M. Lievens: "*Medical and technical usefulness of measurement of kappa and lambda immunoglobulin light chains in serum with an M-component*" - J Clin Chem Clin Biochem 1989; 27; 519-23.
(2) EDMA Labelling Task Force: "*EDMA Symbols for IVD Reagents and Components - Revision, October 2009*".

TEXT REVISION DATE

6th April 2020.