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CE IVD

EN

INSTRUCTIONS FOR USE

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

3diag - KAP - TIA

KAPPA Light Chains (serum+urine)
for Turbidimetry
REF **TD-42771**

INTENDED USE

Quantitative determination of KAPPA Light Chains (bound and free) (KAP), in human serum and urine, by turbidimetric method in automatic Clinical Chemistry Analyzers.

PRINCIPLE OF THE METHOD

The specific antibodies (Ab) of the reagent, 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 | KAP
  REF TD-42771-RA ▽ 100 test - 5 ml
  Anti-human KAP antibodies solution.
- Reaction Buffer: BUF | KAP
  REF TD-42771-BF ▽ 100 test - 25 ml
  TRIS Buffer, with PEG.

As a preservative, the reagents contain <0.1% (1 g/l) Sodium Azide (NaN_3).
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.
When inserting a reagent container into the analyzer, it is always recommended to wait a while before using it, in order for its temperature to stabilize.

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.
- 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, provided they are handled with adequate precautions to avoid contamination, 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 340 nm, and accessories: reagent containers, cuvettes, etc..

3diag - KL - CAL SET	REF TD-42772
3diag - U-KL - CAL SET	REF TD-42782
kloneus® - U-FLC - CAL SET	REF TD-42501-U
3diag - KL - CONTROL	REF TD-42774
3diag - U-KL - CONTROL	REF TD-42793
kloneus® - U-FLC - CONTROL	REF TD-42502-U

SAMPLES

- Fresh Serum.
  Samples with presence of fibrin should be centrifuged.
  Do not use hemolyzed, lipemic or contaminated 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 concentration 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 the following stability :

- Serum: Refrigerated/Freezed: 28 days
- Urine: 7 days in refrigerated urine (sample of preference). The sample should always be kept refrigerated.

Specific guidelines[6] establish that it is the responsibility of each laboratory to consult all available references or to carry out its own studies to determine its specific stability criteria.

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 - Serum / Urine

- ①Dispense and mix:
  - Sample/Calibrator/Control: Serum: 10 µl (dilution 1:40)
    Urine: 20 µl (neat)
  - BUF | KAP 250 µl
- ②Incubate a fixed time between 1 and 5 minutes
- ③Read absorbance A1 (Blank) at 340 nm
- ④Dispense and mix:
  - REAG | Ab | KAP Serum: 50 µl
    Urine: 30 µl
- ⑤Incubate a fixed time of about 5 minutes

- ⑥Read absorbance A2 (End Point) at 340 nm
- ⑦Interpolate the absorbance increment (A2-A1) of the samples and controls in the curve obtained with the calibrators

Samples with concentrations higher than the upper limit of the assay range should be analyzed again, diluted manually with Saline, or by programming a larger sample dilution in the analyzer, to recover a value close to the midpoint of the measurement range.

Calibration Parameters - Serum / Urine

- Serum: Use the **3diag - KL - CAL SET**.
- Urine: It is recommended to use the **λoneus® - U-FLC - CAL SET**, constituted by human Free Light Chain (FLC) solutions, or alternatively the **3diag - U-KL - CAL SET**, consisting of human serum solutions containing bound Light Chains.
- If the analyzer allows it, it is recommended to program two replicates of each calibration point.
- The calibrations are Non-linear. For the calculation it is recommended to use a 3rd Order Polynomial, a Logit or a Polygonal adjustment.
- The calibration curves have a limited validity, which depends on the particular conditions of use. The assays should be recalibrated when:
  - a new lot of reagents is used,
  - established internal quality control procedures do not deliver the expected results, or
  - after performing maintenance operations on the analyzer.

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 Light Chains 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 Light Chains, 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 extreme values of Light Chains 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, such as the determination of Free Light Chains (FLC) in serum and urine, serum Immunoglobulins, electrophoretic assays, or the determination of Light Chains at the same time in serum and urine, may be a useful alarm signal in case of obtaining discordant results.

QUALITY CONTROL

To monitor performances, it is recommended to use the controls of the **3diag - KL - CONTROL** for the serum, and the **λoneus® - U-FLC - CONTROL** or the **3diag - U-KL - CONTROL**, depending on the calibrator used, for the urine.

The insertion of internal controls is recommended:

- in each analytical series,
- when using a new reagent kit from the same lot, and
- after performing a calibration.

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 the event that controls do not give the expected results, the following should be done:

- repeat controls,
- if the deviation persists, repeat with new controls,
- if the deviation persists, calibrate again, and
- if the deviation persists, contact the Customer Support Service (✉ support@3diag.com - ☎ +34 93 244 86 79).

As a precaution, until the causes of the deviation have been identified and corrected:

- all reagents should be considered unreliable, and
- sample results should not be validated.

TRACEABILITY

Values are referred to the *European Reference Material ERM-DA470k/IFCC* (*Institute for Reference Materials and Measurements*, *IRMM*), using the *M.M. Lievens* formula[7].

The values of the Free Light Chain calibrators and controls 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.

The bibliography reports reference values of:

- Serum: between 170 and 370 mg/dl[8].
  Reference values for KAP/LAM ratio are between 1.35 and 2.65[8].
- Urine: up to 0,9 mg/dl[5].
  In general, Free Light Chains (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 to be considered as significant.

LIMITATIONS OF THE PROCEDURE

- Hemolyzed, lipemic or turbid samples, which cannot be clarified by centrifugation, should not be used in turbidimetric or nephelometric assays as turbidity and particles can interfere with the determination.
- Samples containing circulating immune complexes (CICs) / heterophilic antibodies can lead to erroneously increased or decreased results in immunoassays. Unexpected or inconsistent results should be confirmed using alternative methods.
- The product must be used as described in these instructions by suitably trained personnel. Any modification made to the assay and its necessary validation is the sole responsibility of the user, as well as the validation on each particular analyzer.
- Samples from internal quality controls other than the recommended one, or from external quality controls, may give different results than those obtained by other methods, due to matrix effects. To evaluate the results it may be necessary to establish specific target values for the method.
- It is well known that immunochemical assays are not suitable for the measurement of paraproteins, due to the possibility of a non-linear response and therefore lack of accuracy and inconsistent results. Furthermore, an immunochemical measurement cannot distinguish between monoclonal and polyclonal proteins. Light Chain concentrations can never be considered as a measure of the monoclonal component.
- When changing the method, it is advisable to carry out additional sequential measurements to establish new baseline values that allow monitoring the evolution of patients.

CLINICAL SIGNIFICANCE

Quantities of Light Chains exceeding normal values or an abnormal KAP/LAM 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 Light Chains as a screening method for the presence of Bence-Jones proteinuria (BJP), that may also be useful in monitoring.

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[9] by the *EDMA* (*European Diagnostic Manufacturers Association*), whose meaning is detailed below.

REAG	Reagent
Ab	Antibody / Antiserum
BUF	Buffer
KAP	KAPPA Light Chains
CAL	Calibrator
H	High
L	Low
KL	Light Chains (Kappa and Lambda)

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: 25th February 2019.

(6) Clinical and Laboratory Standards Institute (CLSI), Doc. GP44-A4, May 2010: "*Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Test; Approved Guideline - Fourth Edition*"

(7) 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.

(8) Siemens Heathcare Diagnostics Products GmbH: "*Sistema BN° II - Protocolos de Ensayo - Versión 2.4*", 2009/03.

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

TEXT REVISION DATE

11th June 2021.

Modifications highlighted in blue.

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INSTRUCTIONS FOR USE

Reagents for professional use,
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3diag - U-KL - CAL SET

Kappa and Lambda Light Chains
Urine - Calibrators (6 lev.)
REF **TD-42782**

INTENDED USE

Elaboration of the calibration curve for the quantitative determination of Kappa and Lambda Light Chains (bound and free) (KAP and LAM), in human urine, by immunochemical methods.

PRINCIPLE OF THE METHOD

The specific antibodies (Ab) of the reagent, 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

- Calibrator Level 1: CAL 1 U-KL
  REF TD-42782-1 CONT 1 ml
- Calibrator Level 2: CAL 2 U-KL
  REF TD-42782-2 CONT 1 ml
- Calibrator Level 3: CAL 3 U-KL
  REF TD-42782-3 CONT 1 ml
- Calibrator Level 4: CAL 4 U-KL
  REF TD-42782-4 CONT 1 ml
- Calibrator Level 5: CAL 5 U-KL
  REF TD-42782-5 CONT 1 ml
- Calibrator Level 6: CAL 6 U-KL
  REF TD-42782-6 CONT 1 ml

The calibrators are human serum solutions, delipidated, filtered by 0.2 µm.
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:

- 3diag - KAP - TIA — REF TD-42771
- 3diag - LAM - TIA — REF TD-42791
- 3diag - U-KL - CONTROL — REF TD-42793

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

Values are referred to the *European Reference Material ERM-DA470k/IFCC* (*Institute for Reference Materials and Measurements, IRMM*), using the *M.M. Lievens* formula[1].

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-KL	Kappa and Lambda Light Chains (B&F) - 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

3rd May 2021.

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INSTRUCTIONS FOR USE

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

3diag - U-KL - CONTROL

Kappa and Lambda Light Chains - Urine
Controls (2 lev.)
REF **TD-42793**

INTENDED USE

Internal controls, with assigned values, for the quantitative determination of Kappa and Lambda Light Chains (bound and free) (KAP and LAM), in human urine, by immunochemical methods.

PRINCIPLE OF THE METHOD

The specific antibodies (Ab) of the reagent 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-KL
  REF TD-42793-H — CONT 1 ml
- Low Control: CONTROL | L | U-KL
  REF TD-42793-L — CONT 1 ml

The controls are solutions of human light chains.
As preservatives, the controls contain <0.1% (1 g/l) Sodium Azide (NaN3), <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.
It is always advisable to bring the controls to room temperature before use.
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:

3diag - KAP - TIA	REF TD-42771
3diag - LAM - TIA	REF TD-42791
3diag - KL - CAL	REF TD-42792

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 the Reagents.

TRACEABILITY

Values are referred to the *European Reference Material ERM-DA470k/IFCC* (*Institute for Reference Materials and Measurements*, IRMM), using the M.M. Lievens formula[1].

(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.

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.

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

CONTROL	Control
H	High
L	Low
U-KL	Kappa and Lambda Light Chains - Urine
CONT	Contents

TEXT REVISION DATE

30th July 2020.