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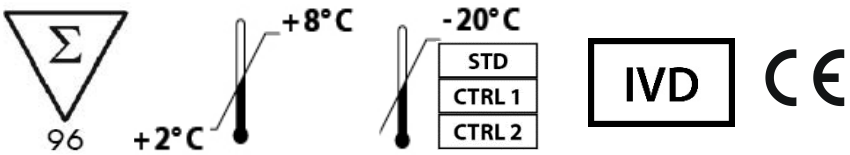
Manual

IDK® Quinolinic acid (QuinA) ELISA

For the in vitro determination of quinolinic acid in urine

Valid from 2019-02-12

REF **K 7736**



IVD CE

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1. INTENDED USE

This ELISA is intended for the quantitative determination of quinolinic acid (pyridine-2,3-dicarboxylic acid) in urine. For *in vitro* diagnostic use only.

2. MATERIAL SUPPLIED

Cat. No.	Label	Kit Components	Quantity
K 7736	PLATE	Microtiter plate, pre-coated	12 x 8 wells
K 7736	STD	Standards, ready-to-use (0, 3, 10, 30, 100, 300 µmol/l)	6 x 200 µl
K 7736	CTRL 1	Control, ready-to-use (see specification for range)	1 x 200 µl
K 7736	CTRL 2	Control, ready-to-use (see specification for range)	1 x 200 µl
K 0006.C.100	WASHBUF A	Wash buffer concentrate, 10x	2 x 100 ml
K 7736	AB	Quinolinic acid antibody, lyophilised	1 vial
K 7736	ABBUF	Antibody dilution buffer, ready-to-use	1 x 8 ml
K 7736	ASYBUF	Assay buffer, ready-to-use	1 x 12 ml
K 7736	CONJ	Conjugate, peroxidase-labelled, ready-to-use	1 x 12 ml
K 0002.15	SUB	Substrate (tetramethylbenzidine), ready-to-use	1 x 15 ml
K 0003.15	STOP	Stop solution, ready-to-use	1 x 15 ml

For reorders of single components, use the catalogue number followed by the label as product number.

3. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultra pure water*
- Calibrated precision pipets and 10-1000 µl single-use tips
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker

- Multi-channel pipets or repeater pipets
- Vortex
- Standard single-use laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 6)

* Immundiagnostik AG recommends the use of ultrapure water (water type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25 °C (≥18.2 MΩ cm).

4. PREPARATION AND STORAGE OF REAGENTS

- To run the assay more than once, ensure that reagents are stored at the conditions stated on the label.
- **Preparation of the wash buffer**: The **wash buffer concentrate (WASHBUF A)** has to be diluted with ultrapure water **1:10** before use (100 ml WASHBUF A + 900 ml ultrapure water), mix well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals have to be redissolved at room temperature or in a water bath at 37 °C. The **WASHBUF A** is stable at **2-8 °C** until the expiry date stated on the label. **Wash buffer** (1:10 diluted WASHBUF A) can be stored in a closed flask at **2-8 °C for 1 month**.
- Store **standards and controls (STD/CTRL)** frozen at **-20 °C**. They are stable at -20 °C until the expiry date stated on the label. Thaw before use in the test and mix well. Re-freeze standards and controls after use.
- **The lyophilised quinolinic acid antibody (AB)** is stable at **2-8 °C** until the expiry date stated on the label. Before use, reconstitute the AB in **6 ml of antibody dilution buffer (ABBUF)**. Allow to dissolve for 10 minutes and mix thoroughly with a vortex-mixer. **Quinolinic acid antibody** (reconstituted AB) **can be stored at 2-8 °C for 2 months.**
- All other test reagents are ready-to-use. Test reagents are stable until the expiry date (see label) when stored at **2-8 °C.**

5. PREPARATION AND STORAGE OF SAMPLES

Storage of samples

Acidified urine samples can be stored for 6 days at room temperature. Non-acidified samples can be stored for 48 h at room temperature or for 4 days at 2-8 °C.

For longer storage keep samples frozen at -20 °C. Avoid repeated thawing and freezing.

Preparation of samples, controls and standards

1.	Add **50 µl standard** (STD)/**control** (CTRL)/**urine sample** in 1.5 ml polypropylene vials.
2.	Add **100 µl assay buffer** (ASYBUF) into each vial (STD/CTRL/samples), mix well.

For analysis, **50 µl** of the prepared standards, controls and samples are used per well.

6. ASSAY PROCEDURE

Principle of the test

This ELISA is designed for the quantitative determination of quinolinic acid. The assay is based on the method of competitive enzyme linked immunoassays.

Samples, standards and controls are incubated in wells of a microtiter plate coated with quinolinic acid (antigen), together with a polyclonal anti-quinolinic acid antibody. The free target antigen in the sample competes with the antigen immobilized on the wall of the microtiter wells for the binding of the polyclonal antibodies.

In the second incubation step, a peroxidase-conjugated antibody is added to each microtiter well to detect the anti-quinolinic acid antibodies. After a washing step to remove the unbound components, the peroxidase substrate tetramethylbenzidine (TMB) is added. Finally, the enzymatic reaction is terminated by an acidic stop solution. The colour changes from blue to yellow and the absorbance is measured in the photometer at 450 nm. The intensity of the yellow colour is inverse proportional to the quinolinic acid concentration in the sample. This means, high antigen concentration in the sample reduces the concentration of antibodies bound to the antigen on the plate and lowers the photometric signal. A dose response curve of the absorbance unit (optical density, OD at 450 nm) vs. concentration is generated, using the values obtained from the standards. Quinolinic acid present in the patient samples is determined directly from this curve.

Test procedure

Bring all **reagents and samples to room temperature** (15-30 °C) and mix well.

Mark the positions of standards/controls/samples on a protocol sheet.

Take as many microtiter strips as needed from the kit. Store unused strips covered with foil at 2-8 °C. Strips are stable until expiry date stated on the label.

For automated ELISA processors, the given protocol may need to be adjusted according to the specific features of the respective automated platform. For further details please contact your supplier or Immundiagnostik AG.

We recommend carrying out the tests in duplicate.

1.	Add **50 µl** of the **prepared standards/controls/samples** into the respective wells of the microtiter plate.
2.	Add **50 µl quinolinic acid antibody** into each well.
3.	Cover the strips tightly and incubate **overnight** (15-24 hours) **at 2-8 °C.**
4.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
5.	Add **100 µl conjugate** into each well.
6.	Cover the strips and incubate for **1 hour** at room temperature (15-30 °C) on a **horizontal shaker**.
7.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
8.	Add **100 µl substrate** (SUB) into each well.
9.	Incubate for **12-18 min*** at room temperature (15-30 °C) in the **dark.**
10.	Add **100 µl stop solution** (STOP) into each well and mix well.
11.	Determine **absorption immediately** with an ELISA reader at **450 nm** against 620 nm (or 690 nm) as a reference. If no reference wavelength is available, read only at 450 nm. If the extinction of the highest standard exceeds the range of the photometer, absorption must be measured immediately at **405 nm** against 620 nm (690 nm) as a reference.

* The intensity of the colour change is temperature sensitive. We recommend observing the colour change and stopping the reaction upon good differentiation.

7. RESULTS

The following algorithms can be used alternatively to calculate the results. We recommend using the 4 parameter algorithm.

1. **4 parameter algorithm**

   It is recommended to use a linear ordinate for optical density and a logarithmic abscissa for concentration. When using a logarithmic abscissa, the zero standard must be specified with a value less than 1 (e.g. 0.001).

2. **Point-to-point calculation**

   We recommend a linear ordinate for optical density and a linear abscissa for concentration.

3. **Spline algorithm**

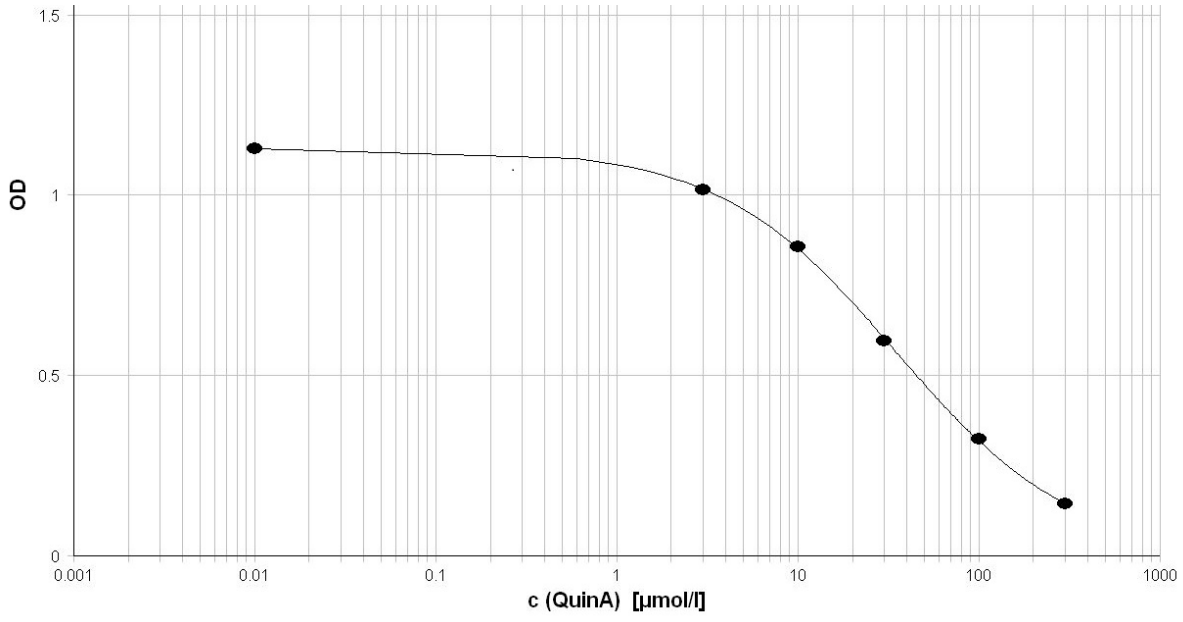
   We recommend a linear ordinate for optical density and a linear abscissa for concentration.

The plausibility of the duplicate values should be examined before the automatic evaluation of the results. If this option is not available with the program used, the duplicate values should be evaluated manually.

Urine samples

No factor is required.

In the following, an example of a standard curve is given Do not use it for the calculation of your results.



8. LIMITATIONS

Samples with concentrations above the measurement range can be further diluted with assay buffer and re-assayed. Please consider this dilution factor when calculating the results.

Samples with concentrations lower than the measurement range cannot be clearly quantified.

The upper limit of the measurement range can be calculated as:

highest concentration of the standard curve × *sample dilution factor to be used*

The lower limit of the measurement range can be calculated as:

Analytical sensitivity × *sample dilution factor to be used*

Analytical sensitivity see chapter "Performance Characteristics".

Biotin interference

Samples containing a biotin concentration of < 1200 ng/ml show a change of the results of ≤ 25 %. Higher concentrations of biotin can lead to falsely low results. Patients taking > 5 mg biotin per day should wait at least 24 hours after taking biotin to have their samples collected. Results of patients taking biotin supplements or receiving a high-dose biotin therapy should generally be interpreted along with the total clinical picture.

9. QUALITY CONTROL

Immundiagnostik AG recommends the use of external controls for internal quality control, if possible.

Control samples should be analysed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the patient samples may not be valid if within the same assay one or more values of the quality control samples are outside of the acceptable limits.

Reference Range

Based on internal studies with urine samples of apparently healthy persons, a median of 2.2 µmol/mmol creatinine was measured (n = 61). The range between the 10^{th} and 90^{th} percentile was 1.2 - 3.9 µmol/mmol creatinine.

We recommend each laboratory to establish its own reference range.

10. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

Intra-assay (n = 8)

sample	quinolinic acid [µmol/l]	CV [%]
1	32.03	7.1
2	66.99	5.9

Inter-assay (n = 10)

sample	quinolinic acid [µmol/l]	CV [%]
1	74.05	4.3
2	49.34	5.8

Spiking recovery

3 urine samples were spiked with different quinolinic acid concentrations and measured in this assay. The mean recovery rate was 101.0 % (n = 2).

sample	spike [µmol/l]	expected [µmol/l]	measured [µmol/l]	recovery [%]
A			35.03	
	20	55.03	56.94	103.47
	40	75.03	75.21	100.24
B			54.45	
	20	74.45	75.61	101.56
	40	94.45	98.39	104.17
C			26.97	
	20	46.97	45.20	96.22
	40	66.97	67.32	100.53

Dilution recovery

2 urine samples were diluted with assay buffer and measured in this assay. The mean recovery rate was 101.0 % (n = 2).

sample	dilution	expected [µmol/l]	measured [µmol/l]	recovery [%]
A	1:3		70.7	
	1:4	53.0	56.3	106.2
	1:6	35.4	33.5	94.6
B	1:3		49.0	
	1:4	36.8	36.2	98.6
	1:6	24.5	25.6	104.6

Analytical sensitivity

The zero-standard was measured 48 times. The detection limit was set as B_0 - 2 SD and estimated to be 1.7 µmol/l.

Specificity

The specificity of the antibody was tested by measuring the cross-reactivity against a range of compounds with structural similarity to quinolinic acid. The specificity is calculated in percent in relation to the quinolinic acid binding activity.

L-kynurenine	0 %
Kynurenic acid	0 %
Serotonin	0 %
L-tryptophan	0 %
L-OH-kynurenine	0 %
Indole-3-acetic acid	0 %

11. PRECAUTIONS

- All reagents in the kit package are for *in vitro* diagnostic use only.
- Human materials used in kit components were tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.
- Kit reagents contain sodium azide or ProClin as bactericides. Sodium azide and ProClin are toxic. Substrates for the enzymatic color reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes
- The stop solution consists of sulfuric acid, which is a strong acid. Although diluted, it still must be handled with care. It can cause burns and should be

handled with gloves, eye protection, and appropriate protective clothing. Any spills should be wiped up immediately with copious quantities of water. Do not breathe vapour and avoid inhalation.

12. TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay. Furthermore, we recommend not assembling wells of different microtiter plates for analysis, even if they are of the same batch.
- Control Samples should be analysed with each run.
- Reagents should not be used beyond the expiration date stated on the kit label.
- Substrate solution should remain colourless until use.
- To ensure accurate results proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- Do not mix plugs and caps from different reagents.
- The assay should always be performed according to the enclosed manual.

13. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- The guidelines for medical laboratories should be followed.
- *IDK*® is a trademark of Immundiagnostik AG.
- Incubation time, incubation temperature, and pipetting volumes of the different components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from incorrect use.
- Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product. The product should be sent to Immundiagnostik AG along with a written complaint.

14. REFERENCES

General literature

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5. Noakes RR. Effects of tranilast on the urinary excretion of kynurenic and quinolinic Acid under conditions of L tryptophan loading. *International journal of tryptophan research : IJTR*. 2013;6:67-71. doi:10.4137/IJTR.S12797
6. Steiner J, Walter M, Gos T, et al. Severe depression is associated with increased microglial quinolinic acid in subregions of the anterior cingulate gyrus: Evidence for an immune-modulated glutamatergic neurotransmission? *Journal of Neuroinflammation*. 2011;8(1):94. doi:10.1186/1742-2094-8-94

Literature using Immundiagnostik IDK® Quinolinic acid ELISA

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Used symbols:

 Temperature limitation

Catalogue Number

In Vitro Diagnostic Medical Device

To be used with

 Manufacturer

Contains sufficient for <n> tests

Lot number

 Use by

 Attention

 Consult instructions for use

Consult specification data sheet