



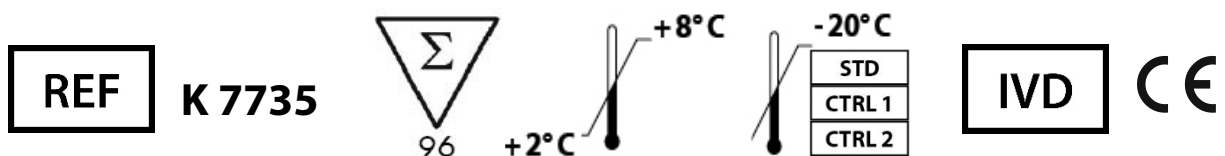
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Manual

IDK® Kynurenic acid (KynA) ELISA

For the in vitro determination of kynurenic acid in urine

Valid from 2019-07-17



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1. INTENDED USE

This ELISA is intended for the quantitative determination of kynurenic acid in urine. For *in vitro* diagnostic use only.

2. MATERIAL SUPPLIED

Cat. No.	Label	Kit Components	Quantity
K 7735	PLATE	Microtiter plate, pre-coated	12 x 8 wells
K 7735	STD	Standards, ready-to-use (0, 1.5, 4.5, 15, 45, 150 µmol/l)	6 x 200 µl
K 7735	CTRL 1	Control, ready-to-use (see specification for range)	1 x 200 µl
K 7735	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 7735	AB	Kynurenic acid antibody, peroxidase-labelled, ready-to-use	1 x 6 ml
K 7735	ASYBUF	Assay buffer, ready-to-use	2 x 30 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

- Ultrapure 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.
- 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 4 days at room temperature. Non-acidified samples can be stored for 24 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 500 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 kynurenic 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 kynurenic acid (antigen), together with a peroxidase labeled polyclonal anti-kynurenic acid antibody. During the incubation period the free target antigen in the sample competes with the antigen immobilized on the wall of the microtiter wells for the binding of the peroxidase-labelled polyclonal 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 kynurenic 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. Kynurenic 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 kynurenic 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 substrate (SUB) into each well.
6.	Incubate for 12-18 min* at room temperature (15-30 °C) in the dark .
7.	Add 100 µl stop solution (STOP) into each well and mix well.
8.	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

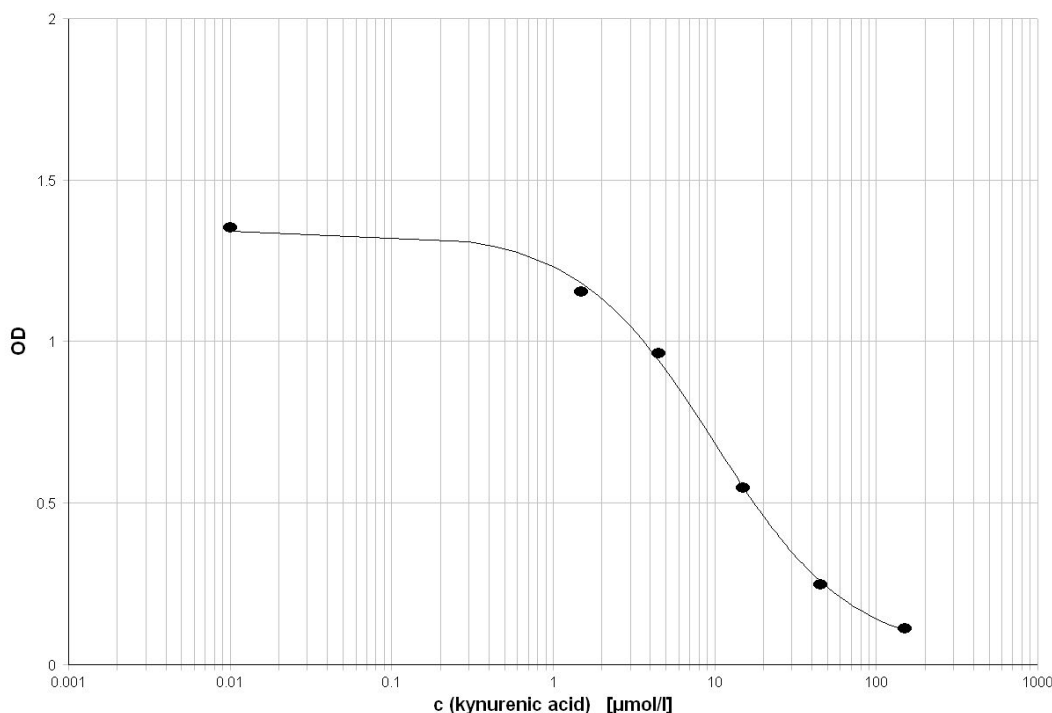
We recommend a linear ordinate for optical density and a linear abscissa for concentration.

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 < 44 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 1.8 µmol/mmol creatinine was measured (n = 61). 90 % of the values were found to be higher than or equal to 1.3 µmol/mmol creatinine.

We recommend each laboratory to establish its own reference range.

10. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

Intra-assay (n = 24)

sample	kynurenic acid [µmol/l]	CV [%]
1	28.8	5.7
2	26.9	8.0

Inter-assay (n = 7)

sample	kynurenic acid [µmol/l]	CV [%]
1	15.9	11.4
2	33.0	9.8

Spiking recovery

3 urine samples were spiked with different kynurenic acid concentrations and measured in this assay. The mean recovery rate was 95.8 % (n = 2).

sample	spike [µmol/l]	expected [µmol/l]	measured [µmol/l]	recovery [%]
A			21.71	
	10	31.71	33.69	106.2
	20	41.71	44.60	106.9
B			18.73	
	10	28.73	28.32	98.6
	20	38.73	33.79	87.3
C			17.18	
	10	27.18	24.28	89.3
	20	37.18	32.20	86.6

Dilution recovery

3 urine samples were diluted with assay buffer and measured in this assay. The mean recovery rate was 106.8 % (n = 2).

sample	dilution	expected [µmol/l]	measured [µmol/l]	recovery [%]
A			10.64	
	1:2	5.32	6.00	112.7
	1:3	3.55	3.88	109.4
B			4.04	
	1:2	2.02	1.94	95.9
	1:3	1.35	1.39	103.0
C			44.46	
	1:2	22.23	22.23	100.0
	1:3	14.82	17.76	119.8

Analytical sensitivity

The zero-standard was measured 48 times. The detection limit was set as $B_0 - 2 SD$ and estimated to be 0.3 $\mu\text{mol/l}$.

Specificity

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

Quinolinic acid	< 0.003 %
Serotonin	< 0.04 %
Indole-3-acetic acid	< 0.01 %
L-tryptophan	< 0.14 %
L-OH-kynurenine	< 0.16 %
L-kynurenine	< 0.11 %

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

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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		