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Instructions for use

Kynurenic acid ELISA

REF **IS I-0200R**

RUO

1. Intended use and principle of the test

Enzyme Immunoassay for the quantitative determination of Kynurenic acid in serum samples.
After extraction and derivatization Kynurenic acid is quantitatively determined by ELISA.
The competitive ELISA uses the microtiter plate format. The antigen is bound to the solid phase of the microtiter plate. The processed standards, controls and samples and the solid phase bound analyte compete for a fixed number of antiserum binding sites. When the system is in equilibrium, free antigen and free antigen-antiserum complexes are removed by washing. The antibody bound to the solid phase is detected by an anti-rabbit IgG-peroxidase conjugate using TMB as a substrate. The reaction is monitored at 450 nm.
Quantification of unknown samples is achieved by comparing their absorbance with a reference curve prepared with known standards.

2. Procedural cautions, guidelines, warnings and limitations

2.1 Procedural cautions, guidelines and warnings

(1) This kit is intended for professional use only. Users should have a thorough understanding of this protocol for the successful use of this kit. Only the test instruction provided with the kit is valid and has to be used to run the assay. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
(2) This assay was validated for certain types of samples as indicated in *Intended Use* (please refer to Chapter 1). Any off-label use of this kit is in the responsibility of the user and the manufacturer cannot be held liable.
(3) The principles of Good Laboratory Practice (GLP) have to be followed.
(4) In order to reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves and protective glasses where necessary.
(5) All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
(6) For dilution or reconstitution purposes, use deionized, distilled or ultra-pure water.
(7) The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch with desiccant and used in the frame provided.
(8) Duplicate determination of sample is highly recommended to be able to identify potential pipetting errors.
(9) Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials and devices are prepared ready at the appropriate time.
(10) Incubation times do influence the results. All wells should be handled in the same order and time intervals.
(11) To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
(12) A standard curve must be established for each run.
(13) The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report.
(14) Do not mix kit components with different lot numbers within a test and do not use reagents beyond expiry date as shown on the kit labels.
(15) Avoid contact with Extraction reagent, Extraction wash buffer and Stop Solution containing acid solutions. It may cause skin irritation and burns. In case of contact with eyes or skin, rinse off immediately with water.
(16) TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them.
(17) For information on hazardous substances included in the kit please refer to Safety Data Sheet (SDS). The Safety Data Sheet for this product is made available directly on the website of the manufacturer or upon request.
(18) The expected reference values reported in this test instruction are only indicative. It is recommended that each laboratory establishes its own reference intervals.
(19) The results obtained with this test kit should not be taken as the sole reason for any therapeutic consequence but have to be correlated to other diagnostic tests and clinical observations.
(20) Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

2.2 Limitations

Any inappropriate handling of samples or modification of this test might influence the results.
Samples containing precipitates or fibrin strands or which are haemolytic or lipemic might cause inaccurate results.

3. Storage and stability

Store the unopened reagents at 2 - 8 °C until expiration date. Do not use components beyond the expiry date indicated on the kit labels. Once opened the reagents are stable for 1 month when stored at 2 – 8 °C. Once the resealable pouch has been opened, care should be taken to close it tightly with desiccant again.

4. Materials

4.1 Contents of the kit

BA D-0090 FOILS **Adhesive Foil** - Ready to use
Contents: Adhesive Foils in a resealable pouch
Volume: 1 x 4 foils

BA R-8318 EXTRACT-PLATE 96 **Extraction Plate** - Ready to use
Contents: 1 x 96 well plate, in a resealable pouch

BA E-0030 WASH-CONC 50x **ELISA Wash Buffer Concentrate** - Concentrated 50x
Contents: Buffer with a non-ionic detergent and physiological pH
Volume: 1 x 20 mL/vial, light purple cap

BA E-0040 CONJUGATE **Enzyme Conjugate** - Ready to use
Contents: Goat anti-rabbit immunoglobulins conjugated with peroxidase
Volume: 1 x 12 mL/vial, red cap

BA E-0055 SUBSTRATE **Substrate** - Ready to use
Contents: Chromogenic substrate containing tetramethylbenzidine, substrate buffer and hydrogen peroxide
Volume: 1 x 12 mL/black vial, black cap

BA E-0080 STOP-SOLN **Stop Solution** - Ready to use
Contents: 0.25 M sulfuric acid
Volume: 1 x 12 mL/vial, light grey cap
Hazards identification:

H290 May be corrosive to metals.

IS I-0231 TLT KYNA **Kynurenic acid Microtiter Strips** - Ready to use
Contents: 1 x 96 well (12x8) antigen precoated microwell plate in a resealable pouch with desiccant

IS I-0210 AS KYNA CONC 2x **Kynurenic acid Antiserum Concentrate** - Concentrated 2x
Contents: Rabbit anti-kynurenic acid antibody, blue coloured
Volume: 1 x 7 mL/vial, blue cap

IS I-0211 AS KYNA DILUENT **Kynurenic acid Antiserum Diluent** - Ready to use
Volume: 1 x 7 mL/vial, orange cap

IS I-0212 EXTRACT-REAG **Extraction Reagent** - Ready to use
Contents: Buffer with acidic pH
Volume: 1 x 4 mL/vial, dark green cap
Hazards identification :

H290 May be corrosive to metals.

IS I-0213 EXTRACT-WASH **Extraction Wash Buffer** - Ready to use
Contents: Buffer with acidic pH
Volume: 1 x 35 mL/vial, white cap

IS I-0214 CLEAN-BUFF **Cleaning Buffer** - Ready to use

Contents: Buffer with acidic pH

Volume: 1 x 35 mL/vial, light yellow cap

IS I-0216 ASSAY-BUFF **Assay Buffer** - Ready to use

Contents: Buffer with acidic pH

Volume: 1 x 35 mL/vial, dark blue cap

Hazards identification:

H290 May be corrosive to metals.

IS I-0215 ACYL-REAG **Acylation Reagent** - Lyophilized

Volume: 3 vials, dark green cap

BA E-2211 ACYL-BUFF **Acylation Buffer** - Ready to use

Contents: 2-(N-Morpholino)ethanesulfonic acid (MES) buffer

Volume: 2 x 30 mL/vial, brown cap

Standards and **Controls** - Ready to use

Cat. no.	Component	Colour/Cap	Concentration ng/mL	Concentration nmol/L	Volume/ Vial
IS I-0201	STANDARD A	white	0	0	4 mL
IS I-0202	STANDARD B	light yellow	1.89	10	4 mL
IS I-0203	STANDARD C	orange	4.73	25	4 mL
IS I-0204	STANDARD D	dark blue	11.82	62,5	4 mL
IS I-0205	STANDARD E	light grey	29.51	156	4 mL
IS I-0206	STANDARD F	black	73.97	391	4 mL
IS I-0251	CONTROL 1	light green	Refer to QC-Report for expected value and acceptable range!		4 mL
IS I-0252	CONTROL 2	dark red			4 mL

Conversion: Kynurenic acid (ng/mL) x 5.286 = Kynurenic acid (nmol/L)

Contents: Buffer with non-mercury stabilizer, spiked with defined quantity of Kynurenic acid

4.2 Additional materials and equipment required but not provided in the kit

- Calibrated precision pipettes to dispense volumes between 10 – 300 µL; 15 mL; 6 mL
- Microtiter plate washing device (manual, semi-automated or automated)
- ELISA reader capable of reading absorbance at 450 nm and if possible 620 - 650 nm
- Microtiter plate shaker (shaking amplitude 3 mm; approx. 600 rpm)
- Absorbent material (paper towel)
- Water (deionized, distilled or ultra-pure)
- Vortex mixer
- 15mL polypropylene tube

5. Sample collection and storage

Serum

Collect blood by venipuncture (MonovetteTM or VacuetteTM for serum), allow to clot, and separate serum by centrifugation according to manufacturer's instructions. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.
Haemolytic and lipemic samples should not be used for the assay.
Storage: up to 48 hours at 2 - 8 °C, for longer period (up to 6 month) at -20 °C.
Repeated freezing and thawing should be avoided.

6. Test procedure

Allow all reagents and samples to reach room temperature and mix thoroughly by gentle inversion before use. Duplicate determinations are recommended. It is recommended to number the strips of the microwell plate before usage to avoid any mix-up.

The binding of the antisera and of the enzyme conjugate and the activity of the enzyme are temperature dependent, and the absorbance values may vary if a thermostat is not used. The higher the temperature, the higher the extinction values will be. Corresponding variations also apply to the incubation times. The optimal temperature during the Enzyme Immunoassay is between 20 – 25 °C.

6.1 Preparation of reagents

ELISA Wash Buffer
Dilute the 20 mL Wash Buffer Concentrated with water (deionized, distilled or ultra-pure) to a final volume of 1000 mL.
Storage: 1 month at 2 – 8 °C

Acylation Reagent
Reconstitute 1 vial of Acylation reagent – just before use – with 15 mL of Acylation Buffer. Vortex mix until the Acylation Reagent has dissolved completely.
Once prepared, this solution is not stable and can not be re-used.

KYNA Antiserum (AS KYNA)
Calculate the required amount of KYNA Antiserum and prepare – just before use – by mixing equal volumes (1:1) of AS KYNA CONC 2X with AS KYNA DILUENT in a polypropylene tube.
Once prepared, this solution is not stable and can not be re-used.

6.2 Extraction

1.	Pipette **300 µL** of **Assay Buffer** into all wells of the Extraction plate. Incubate the plate for **5 min** at **RT** on a **shaker** (approx. 500 rpm). Empty plate and blot dry by tapping the inverted plate on absorbent material.
2.	Pipette **25 µL** of the **Extraction Reagent** to all wells of the **Extraction plate**.
3.	Add **50 µL** of the **standards, controls** and **samples** into the appropriate wells of the **Extraction plate.**
4.	Cover the plate with **Adhesive Foil** and incubate **1h** at **RT** on a **shaker** (approx. 500 rpm).

6.3 Derivatization

1.	Remove the foil. Discard the contents of the wells. Wash the Extraction plate **1 x** by adding **300 µL** of **Cleaning buffer**, **discarding** the content and **blotting dry** by tapping the inverted plate on absorbent material.
2.	In a second time, wash the Extraction plate **1 x** by adding **300 µL** of **Extraction Wash buffer**, **discarding** the content and **blotting dry** by tapping the inverted plate on absorbent material.
3.	Pipette **140 µL** of the **Acylation Reagent** (refer to 6.1.) into all wells and mix shortly.
4.	Cover the plate with **Adhesive foil** and incubate **90 min** at **37°C**.
5.	**Use 50 µL for the ELISA!**

6.4 Kynurenic acid ELISA

1.	Mix by stirring on a shaker 2 min at 500 rpm to homogenize the medium before pipetting.
2.	Pipette **50 µL** of the **prepared standards, controls and samples** into the appropriate wells of the **Kynurenic acid Microtiter Strips.**
3.	Pipette **100 µL** of the **KYNA Antiserum** (refer to 6.1.) into all wells and mix shortly.
4.	Cover the plate with **Adhesive Foil** and incubate for **15 – 20 h** (overnight) at **2 – 8 °C**.
5.	Remove the foil. Discard or aspirate the contents of the wells. Wash the plate **4 x** by adding **300 µL** of **ELISA Wash Buffer**, **discarding** the content and **blotting dry each time** by tapping the inverted plate on absorbent material.
6.	Pipette **100 µL** of the **Enzyme Conjugate** into all wells.
7.	Incubate for **30 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 500 rpm).
8.	Discard or aspirate the contents of the wells. Wash the plate **4 x** by adding **300 µL** of **ELISA Wash Buffer**, discarding the content and **blotting dry each time** by tapping the inverted plate on absorbent material.
9. ⚠	Pipette **100 µL** of the **Substrate** into all wells and incubate for **20 - 30 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 500 rpm). ***Avoid exposure to direct sunlight!***

10.	Add **100 µL** of the **Stop Solution** to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution.
11.	**Read** the **absorbance** of the solution in the wells within 10 minutes, using a microplate reader set to **450 nm** (if available a reference wavelength between 620 nm and 650 nm is recommended).

7. Calculation of results

Measuring range	**Kynurenic acid**
	10 – 391 nM

The calibration curve is obtained by plotting the absorbance readings (calculate the mean absorbance) of the standards (linear, y-axis) against the corresponding standard concentrations (logarithmic, x-axis).
Use non-linear regression for curve fitting (e.g. spline, 4- parameter, akima).

⚠ *This assay is a competitive assay. This means: the OD-values are decreasing with increasing concentrations of the analyte. OD-values found below the standard curve correspond to high concentrations of the analyte in the sample.*

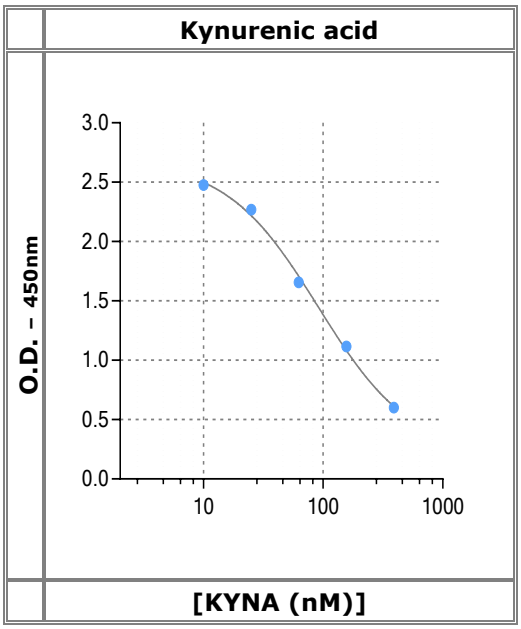
The concentrations of the samples and controls can be read directly from the standard curve.

7.1 Quality control

The confidence limits of the kit controls are indicated on the QC-Report.

7.2 Typical standard curve

⚠ *Example, do not use for calculation!*



8. Assay characteristics

Analytical Sensitivity (Limit of Detection)	**Kynurenic acid**
	2,8 nM

	Substance	**Cross Reactivity (%)**
Analytical Specificity	Kynurenic acid	100
	Quinolinic acid	<0.1
	Xanthurenic acid	<0.1
	Kynurenine	<1
	Picolinic acid	<0.1
	3Hydroxy-Anthranilic acid	<0.1

(Cross Reactivity)	Quinaldic acid	<0.1

Intra-Assay Precision			
Serum sample	Mean (nM)	SD (nM)	CV (%)
1 (n = 8)	55	4	8
2 (n = 8)	96	9	10
3 (n = 8)	137	10	7

Linearity	Serum samples (Serial dilution up to 1:32)	Range Linearity (%)	Mean Linearity (%)
	1	76 – 100	86
	2	74 - 105	87

Recovery	Serum samples	Range Recovery (%)	Mean Recovery (%)
	1	82 – 89	86
	2	89 - 91	90

Method Comparison: ELISA vs LC-MS/MS	Serum samples	$[KYNA]_{ELISA} = 1{,}024*[KYNA]_{LC\text{-}MS/MS} + 0{,}3704$	$R^2 = 0{,}9902$ N = 40

⚠ ***For updated literature or any other information please contact your local supplier.***

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code	RUO	For research use only!
	Consult instructions for use	CONT	Content		
	Caution	REF	Catalogue number		