Version 26-04/24

User's Manual

Quinolinic Acid ELISA Kit

REF **DEIASL166**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The Quinolinic Acid ELISA kit is optimized for the quantitation of Quinolinic Acid (QUIN or QA) within serum and plasma samples.

General Description

Quinolinic acid (QA) is a metabolite produced along the Kynurenine Pathway, which converts the aminoacid Tryptophan to NAD+, a co-factor of many enzymatic reactions.

Principles of Testing

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.

Reagents And Materials Provided

Symbol	Component	Quantity
FOIL	**Adhesive Foil** - Ready to use For covering of Microtiter Plate during incubation	1 x 4
REAC-PLATE	**Reaction Plate** - Ready to use 1 x 96 well plate, in a resealable pouch	96 wells
WASH-CONC 50x	**Wash Buffer, Concentrate 50x** Buffer with a non-ionic detergent and physiological pH	1 x 20 mL
CONJUGATE	**Enzyme Conjugate** - Ready to use Goat anti-rabbit immunoglobulins conjugated with peroxidase	1 x 12 mL
SUBSTRATE	**Substrate** - Ready to use Chromogenic substrate containing tetramethylbenzidine, substrate buffer and hydrogen peroxide	1 x 12 mL
STOP-SOLN	**Stop Solution** - Ready to use 0.25 M sulfuric acid	1 x 12 mL
MICROPLATE	**Quinolinic acid Microtiter Strips** - Ready to use Antigen precoated microwell plate in a resealable pouch with desiccant	1 x 96 wells (12x8)
AS QA CONC 2x	**Quinolinic acid Antiserum Concentrate 2x** Rabbit anti-Quinolinic acid antibody, blue coloured	1 x 4 mL
AS QA DILUENT	**Quinolinic acid Antiserum Diluent** - Ready to use	1 x 4 mL
REAC-DILUENT	**Reaction diluent** - Ready to use	1 x 4 mL
ACYL-REAG	**Acylation Reagent** – Lyophilized	3 vials
ACYL-BUFF	**Acylation buffer** - Ready to use 2-(N-Morpholino)ethanesulfonic acid (MES) buffer	2 x 30 mL

Standards and Controls - Ready to use

Component	Concentration ng/mL	Concentration nmol/L	Volume/ Vial
STANDARD A	0	0	4 mL
STANDARD B	25.08	150	4 mL
STANDARD C	75.25	450	4 mL
STANDARD D	225.75	1350	4 mL
STANDARD E	677.26	4050	4 mL
STANDARD F	2031.77	12150	4 mL
CONTROL 1	Refer to QC-Report for expected value and acceptable range!		4 mL
CONTROL 2			4 mL

Conversion: Quinolinic acid (ng/mL) x 5.98 = Quinolinic acid (nmol/L)

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

Materials Required But Not Supplied

- 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 tubes

Storage

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.

Specimen Collection And Preparation

Serum and plasma (Heparin)

Collect blood by venipuncture (MonovetteTM or VacuetteTM for serum or Heparin plasma), allow to clot, and separate serum or plasma 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.

Reagent Preparation

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 12 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.

QA Antiserum (AS QA)

Calculate the required amount of QA Antiserum and prepare – just before use – by mixing equal volumes (1:1) of AS QA CONC 2X with AS QA DILUENT in a polypropylene tube.

Once prepared, this solution is not stable and can not be re-used.

Assay Procedure

Notes: 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.

Derivatization

1. Pipette 25 µL of the standards, controls and samples into the appropriate wells of the Reaction plate.
2. Add 25 µL of Reaction Diluent into all wells and mix shortly.
3. Add 100 µL of the Acylation Reagent into all wells and mix shortly.
4. Cover the plate with Adhesive foil and incubate 2 hours at 37°C. 5. Use 40 µL for the ELISA!

ELISA Procedure

1. Mix shortly (2min on a shaker at 500 rpm) and pipette 40 µL of the prepared standards, controls and samples into the appropriate wells of the Quinolinic acid Microtiter Strips.
2. Pipette 50 µL of the QA Antiserum into all wells and mix shortly.
3. Cover the plate with Adhesive Foil and incubate for 15 – 20 h (overnight) at 2 – 8 °C.
4. 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.
5. Pipette 100 µL of the Enzyme Conjugate into all wells.
6. Incubate for 30 min at RT (20 – 25 °C) on a shaker (approx. 500 rpm).
7. 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.

8. 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!
9. Add 100 µL of the Stop Solution to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution.
10. 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).

Quality Control

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

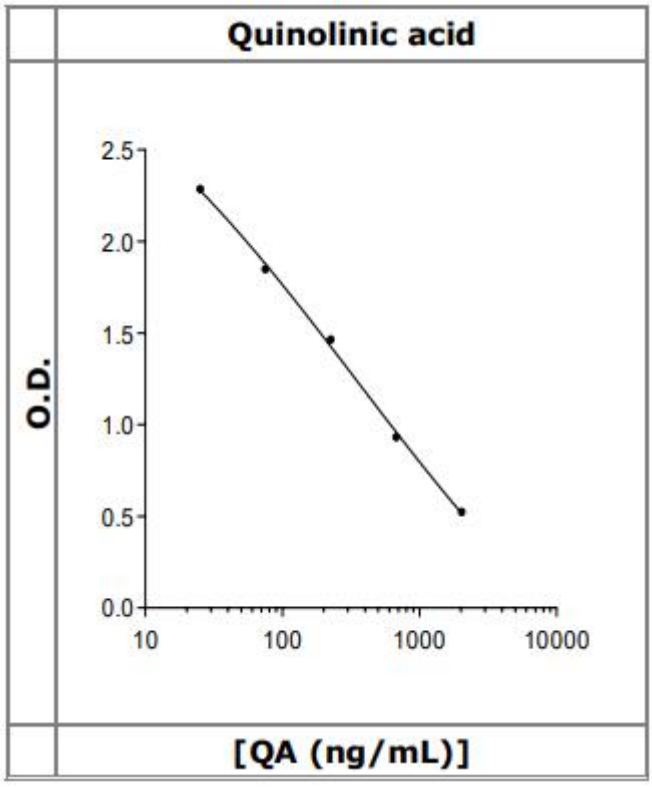
Typical Standard Curve

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.

Example, do not use for calculation!



Precision

Intra-Assay				Inter-Assay			
Serum Sample	Mean (ng/mL)	SD (ng/mL)	CV (%)	Serum Sample	Mean (ng/mL)	SD (ng/mL)	CV (%)
1 (n = 12)	141	16	11.4	1 (n = 10)	70	12	17.0
2 (n = 12)	220	23	10.3	2 (n = 10)	124	21	17.2
3 (n = 12)	341	33	9.5	3 (n = 10)	238	32	13.6

Detection Range

25 - 2000 ng/mL

Sensitivity

< 6 ng/mL

Specificity

No significant cross-reactivity was observed with Quinolinic Acid analogs such as Kynurenic acid, Xanthurenic acid, Kynurenine, Picolinic acid, 3Hydroxy-Anthranilic Acid.

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

Linearity

	Serum samples	Range Linearity (%)	Mean Linearity (%)	Serial dilution up to
Linearity	1	81-117	101	1:16
	2	93-110	98	1:16
	3	100-121	108	1:16

Recovery

	Serum samples	Range Recovery (%)	Mean Recovery (%)	
Recovery	1	108 – 118	112	% Recovery after spiking
	2	100 – 115	109	
	3	101 – 113	107	

Precautions

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 "Sample Preparation". 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 Stop Solution containing 0.25 M H_2SO_4. 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.

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.