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Instructions for use

Kynurenine ELISA

REF **IB89190R**

2°C – 8°C

Σ 96

RUO

For research use only – Not for use in diagnostic procedures

Table of contents

1. Introduction

1.1 Intended use and principle of the test

Enzyme immunoassay for the quantitative determination of L-kynurenine in serum and EDTA-plasma samples to evaluate L-kynurenine homeostasis.
During acylation, kynurenine is activated at 37 °C and subsequently coupled to a protein.
The subsequent competitive ELISA uses the microtiter plate format. The antigen is bound to the solid phase of the microtiter plate. The analyte concentrations of the acylated standards, controls and samples compete with the solid phase bound analyte concentrations for a fixed number of antibody binding sites. After the system is in equilibrium, free antigen and free antigen-antibody 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 resulting in a colour reaction. The reaction is monitored at a wavelength of 450 nm.
Quantification of unknown samples is achieved by comparing their absorbance with a reference curve prepared with known standard concentrations. Manual processing of the ELISA is recommended. The use of automatic laboratory equipment is the responsibility of the user.
This product is not intended to clinical diagnoses.

1.2 Background

Kynurenine is a non-proteinogenic amino acid that is produced as a metabolic intermediate during the degradation of tryptophan [1-5]. The degradation of tryptophan is catalyzed by the inducible enzyme indolamine-2,3-dioxygenase (IDO). The product is kynurenine [4, 6-8]. Cytokines, in particular interferon-γ [5, 9, 10], influence the activity of the IDO, so that is why the kynurenine path is closely linked to the immune system [9, 11]. Kynurenine can be further converted to neuroprotective kynurenic acid, but also to neurotoxic quinolinic acid [6, 11].

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 must be used to run the assay. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
(2) The principles of Good Laboratory Practice (GLP) must be followed.
(3) In order to reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves and protective glasses where necessary.
(4) All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. For dilution or reconstitution purposes, use deionized, distilled, or ultra-pure water. Avoid repeated freezing and thawing of reagents and specimens.
(5) The microplate contains snap-off strips. Unused wells must be stored at 2 – 8 °C in the sealed foil pouch with desiccant and used in the frame provided. Microtiter strips which are removed from the frame for usage should be marked accordingly to avoid any mix-up.
(6) Duplicate determination of sample is highly recommended.
(7) Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials, and devices are prepared for use at the appropriate time.
(8) Incubation times do influence the results. All wells should be handled in the same order and time intervals.
(9) To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
(10) A standard curve must be established for each run.
(11) The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report provided with the kit.
(12) 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.
(13) Avoid contact with 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.
(14) 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. Rinse contaminated items before reuse.
(15) For information about 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.

(16) Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.
(17) In case of any severe damage to the test kit or components, the manufacturer has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components must not be used for a test run. They must be stored properly until the manufacturer decides what to do with them. If it is decided that they are no longer suitable for measurements, they must be disposed of in accordance with national regulations.

2.2 Limitations

Any inappropriate handling of samples or modification of this test might influence the results.

2.2.1 Interfering substances and proper handling of specimens

Serum/Plasma

Hemolytic samples (up to 4 mg/ml hemoglobin), icteric samples (up to 0.5 mg/ml bilirubin) and lipemic samples (up to 17 mg/ml triglycerides) have no influence on the assay results.
If the concentrations cannot be estimated and there are doubts as to whether the above limit values for hemolytic, icteric or lipemic samples are complied with, the samples should not be used in the assay.

2.2.2 Drug and food interferences

Following substances (drugs) are able to interfere with the concentration of kynurenine level in the sample through ingestion: efavirenz, ezetimib/simvastatin, hydrocortisone, 4-hydroxybutanoic acid, navoximod, ACE inhibitors (angiotensin-converting enzyme inhibitor) and ARBs (angiotensin II type 1 receptor blockers) can lower the kynurenine level. Alcohol, interferon-alpha and nivolumab, on the other hand, can increase the kynurenine level.

2.2.3 High-Dose-Hook effect

No hook effect was observed in this test.

3. Storage and stability

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

4. Materials

4.1 Contents of the kit

BA D-0024	**REACT-PLATE** **96**	**Reaction Plate** – ready to use
Content:	1 x 96 well plate, empty in a resealable pouch	
BA D-0090	**FOILS**	**Adhesive Foil** – ready to use
Content:	Adhesive foils in a resealable pouch	
Number:	1 x 4 foils	
BA E-0030	**WASH-CONC** **50x**	**Wash Buffer Concentrate** – concentrated 50x
Content:	Buffer with a non-ionic detergent and physiological pH	
Volume:	1 x 20 ml/vial, purple cap	
BA E-0040	**CONJUGATE**	**Enzyme Conjugate** – ready to use
Content:	Goat anti-rabbit immunoglobulins conjugated with peroxidase	
Volume:	1 x 12 ml/vial, red cap	
Description:	Species is goat	
BA E-0055	**SUBSTRATE**	**Substrate** – ready to use
Content:	Chromogenic substrate containing 3,3',5,5'-tetramethylbenzidine, substrate buffer and hydrogen peroxide	
Volume:	1 x 12 ml/vial, black cap	
BA E-0080	**STOP-SOLN**	**Stop Solution** – ready to use
Content:	0.25 M sulfuric acid	
Volume:	1 x 12 ml/vial, grey cap	

BA E-2210	**AS** **KYN**	**Kynurenine Antiserum** – ready to use
Content:	Rabbit anti-kynurenine antibody in buffer with proteins and non-mercury preservative, blue coloured	
Volume:	1 x 6 ml/vial, blue cap	
Description:	Species of antibody is rabbit, species of protein in buffer is bovine	
BA E-2211	**ACYL-BUFF**	**Acylation Buffer** – ready to use
Content:	2-(N-morpholino)ethanesulfonic acid (MES) buffer	
Volume:	1 x 30 ml/vial, brown cap	
BA E-2212	**ACYL-REAG**	**Acylation Reagent** – ready to use
Content:	Acylation reagent in dimethylsulfoxide (DMSO)	
Volume:	1 x 3 ml/vial, white cap	
Hazard pictograms:	 GHS07	
Signal word:	Warning	
Hazardous ingredients:	N'-(ethylcarbonimidoyl)-N,N-dimethylpropane-1,3-diamine monohydrochloride	
Hazard statements:	H317 May cause an allergic skin reaction. H412 Harmful to aquatic life with long lasting effects.	
Precautionary statements:	P280 Wear protective gloves. P302+P352 IF ON SKIN: Wash with plenty of water. P333+P313 If skin irritation or rash occurs: Get medical advice/attention. P501 Dispose of contents/container to an authorised waste collection point.	
BA E-2231	**Ш** **KYN**	**Kynurenine Microtiter Strips** – ready to use
Content:	1 x 96 wells (12x8) antigen precoated microwell plate in a resealable pouch with desiccant	

4.2 Calibration and Controls

Standards and Controls – ready to use

Cat. no.	Component	Colour/ Cap	Concentration [ng/ml] KYN	Concentration [nmol/l] KYN	Volume/ Vial
BA E-2201	**STANDARD** **A**	white	0	0	4 ml
BA E-2202	**STANDARD** **B**	yellow	100	480	4 ml
BA E-2203	**STANDARD** **C**	orange	300	1,440	4 ml
BA E-2204	**STANDARD** **D**	blue	1,000	4,800	4 ml
BA E-2205	**STANDARD** **E**	grey	3,000	14,400	4 ml
BA E-2206	**STANDARD** **F**	black	10,000	48,000	4 ml
BA E-2251	**CONTROL** **1**	green	Refer to QC-Report for expected value and acceptable range.		4 ml
BA E-2252	**CONTROL** **2**	red			4 ml
Conversion:	kynurenine [ng/ml] x 4.8 = kynurenine [nmol/l]				
Content:	TRIS buffer with non-mercury preservatives, spiked with a defined quantity of kynurenine.				

4.3 Additional materials required but not provided in the kit

- Water (deionized, distilled, or ultra-pure)
- Absorbent material (paper towel)

4.4 Additional equipment required but not provided in the kit

- Calibrated precision pipettes to dispense volumes between 10 – 300 µl
- 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)
- Vortex mixer
- Temperature controlled incubator (37 °C) or similar heating device

5. Sample collection, handling and storage

Repeated thawing and freezing of all samples should be avoided! Fasting specimens are advised.

Plasma

Whole blood should be collected by venepuncture into centrifuge tubes containing EDTA as anticoagulant and centrifuge according to manufacturer´s instructions immediately after collection.
Hemolytic, icteric 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 months) at -15 to -30 °C.

Serum

Whole blood should be collected by venepuncture into centrifuge tubes, allow to clot, and separate serum by centrifugation according to manufacturer´s instructions. Do not centrifuge before complete clotting has occurred. Samples of donors receiving anticoagulant therapy may require increased clotting time.
Hemolytic, icteric 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 months) at -15 to -30 °C.

6. Test procedure

Allow all reagents and samples to reach room temperature and mix thoroughly by gentle inversion before use. Number the Reaction Plate and microwell plate (Microtiter Strips which are removed from the frame for usage should be marked accordingly to avoid any mix-up). Duplicate determinations are recommended.
The binding of the antisera and of the enzyme conjugate and the activity of the enzyme are temperature dependent. The higher the temperature, the higher the absorption values will be. Varying incubation times will have similar influences on the absorbance. The optimal temperature during the enzyme immunoassay is between 20 – 25 °C.
If the product is prepared in parts, unused wells in Reaction Plate should be covered to avoid contamination. After preparation, the used wells must be labelled to prevent double use.
During the overnight incubation at 2 – 8 °C with the antiserum, the temperature should be uniform all over the ELISA plate to avoid any drift and edge-effect.

⚠ *The use of a microtiter plate shaker with the following specifications is mandatory: shaking amplitude 3 mm; approx. 600 rpm. Shaking with differing settings might influence the results.*

6.1 Preparation of reagents and further notes

Wash Buffer

Dilute the 20 ml Wash Buffer Concentrate **WASH-CONC** **50x** with water to a final volume of 1000 ml.
Storage: 2 months at 2 – 8 °C

Acylation Reagent

The Acylation Reagent **ACYL-REAG** has a freezing point of 18.5 °C. To ensure that the Acylation Reagent forms a homogenous, crystal-free solution when being used, it must have reached room temperature.

Kynurenine Microtiter Strips

In rare cases residues of the blocking and stabilizing reagent can be seen in the wells as small, white dots or lines. These residues do not influence the quality of the product.

6.2 Preparation of samples – Acylation

1.	Pipette **10 µl** of **standards**, **controls** und **samples** into the respective wells of the **REAC-PLATE 96**.
2.	Add **250 µl** **ACYL-BUFF** to all wells.
3.	Add **25 µl** **ACYL-REAG** to all wells and incubate **1 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm).
4.	Cover the plate with **FOILS** and incubate for **90 min** at **37 °C**.
⚠	Take **20 µl** of the prepared **standards**, **controls** and **samples** for the **Kynurenine ELISA**.

6.3 Kynurenine ELISA

1.	Pipette **20 µl** of the **acylated standards, controls** and **samples** into the appropriate wells of the **W** **KYN**.
2.	Add **50 µl** of the **AS** **KYN** into all wells and mix shortly.
3.	Cover plate with **FOILS** 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 times** by adding **300 µl** of **Wash buffer**, **discarding** the content and **blotting dry each time** by tapping the inverted plate on absorbent material.
5.	Add **100 µl** of the **CONJUGATE** into each well.
6.	Incubate **30 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm).
7.	Discard or aspirate the contents of the wells. Wash the plate **4 times** by adding **300 µl** of **Wash buffer**, **discarding** the content and **blotting dry each time** by tapping the inverted plate on absorbent material.
8.	Add **100 µl** of the **SUBSTRATE** into each well an incubate for **20 – 30 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm). ***Avoid exposure to direct sunlight!***
9.	Pipette **100 µl** of the **STOP-SOLN** into each well and shake the microtiter plate shortly.
10.	**Read** the absorbance of the solution in the wells within 10 min, using a microtiter plate reader set to **450 nm** (if available a reference wavelength between 620 nm and 650 nm is recommended).

7. Calculation of results

Measuring range	Kynurenine
	63.3 – 10,000 ng/ml

The standard curve, which can be used to determine the concentration of the unknown samples, 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) using a concentration of 0.001 ng/ml for Standard A (this alignment is mandatory because of the logarithmic presentation of the data). Use non-linear regression for curve fitting (e.g. 4-parameter, marquardt).

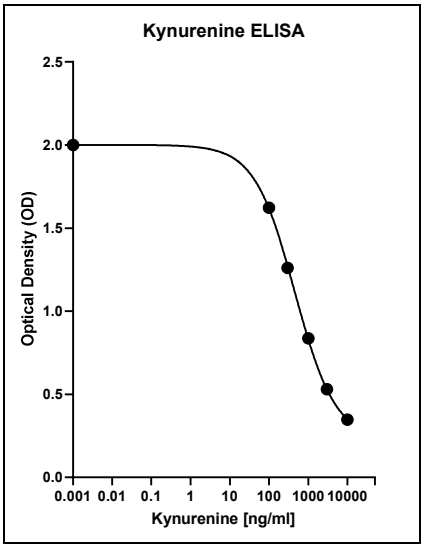
⚠ *This assay is a competitive assay. This means: the OD-values are decreasing with increasing concentrations of the analyte. OD-values found below the standard curve correspond to high concentrations of the analyte in the sample and have to be reported as being positive.*

The concentrations of the samples and controls can be read directly from the standard curve.
Samples found with concentrations higher than the highest standard (Standard F) should be diluted accordingly with Standard A and must be re-assayed.

Conversion:
kynurenine [ng/ml] x 4.8 = kynurenine [nmol/l]

7.1 Typical standard curve

⚠ *Example: Do not use for calculation!*



8. Control samples

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

9. Assay characteristics

9.1 Performance data

Analytical Sensitivity	
Limit of Blank (LOB)	32.2 ng/ml
Limit of Detection (LOD)	45.7 ng/ml
Limit of Quantification (LOQ)	63.3 ng/ml

Analytical Specificity (Cross Reactivity)	
Substance	**Cross Reactivity [%]**
L-Kynurenine	100
5-Hydroxy-DL-Tryptophan, Tyrosine, Phenylalanine, Serotonin, L-Asparagine, Kynurenic acid	0.05
Tryptophan	0.18
3-Hydroxy-DL-Kynurenine	0.3

Precision							
Intra-Assay				**Inter-Assay**			
	Sample	Mean ± SD [ng/ml]	CV [%]		Sample	Mean ± SD [ng/ml]	CV [%]
serum	1	389 ± 48.9	12.6	serum	1	376 ± 66.5	17.7
	2	989 ± 108	11.0		2	889 ± 120	13.5
	3	2,324 ± 256	11.0		3	2,047 ± 203	14.8
plasma	1	400 ± 61.8	15.5	plasma	1	354 ± 44.6	12.6
	2	984 ± 120	12.2		2	867 ± 61.7	7.1
	3	2,230 ± 305	13.8		3	1,916 ± 168	8.8

Lot-to-Lot			
	Sample	Mean ± SD [ng/ml]	CV [%]
Kynurenine in artificial matrix (n = 4)	1	523 ± 37.6	7.2
	2	1,598 ± 127	8.0
Kynurenine in plasma (n = 4)	1	449 ± 21.8	4.9
	2	1,411 ± 211	15.0

Recovery			
	Sample	Mean [%]	Range [%]
serum	1	101	90 – 109
	2	93	90 – 96
	3	109	95 – 118
plasma	1	96	82 – 106
	2	99	90 – 104
	3	103	97 – 110

Linearity			
	Serial dilution up to	Mean [%]	Range [%]
serum	1:128	95	90 – 104
plasma	1:128	94	89 – 102

Method comparison: ELISA vs. XLC-MS/MS	XLC-MS/MS = 0.9x + 71.5; R^2 = 0.9355; n = 30

9.2 Metrological Traceability

The values assigned to the standards and controls of the Kynurenine ELISA are traceable to the weighing.

Standards and Controls	
	Uncertainty [%]
Kynurenine	1.3

Kynurenine ELISA		
plasma	concentration [ng/ml]	Expanded Uncertainty [%] k = 2*
	354	25.3
	867	14.4
serum	concentration [ng/ml]	Expanded Uncertainty [%] k = 2*
	376	35.5
	889	27.1

* This defines an interval about the measured result that will include the true value with a probability of 95%.

10. References/Literature

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

11. Changes

Version	Release Date	Chapter	Change
17.0-r	2022-11-28	All	- The IFU was revised according to the IVDR regulation (EU) 2017/746
		1.	- Introduction
		2.1	- Procedural notes, guidelines and warnings
		2.2.1	- Interfering substances
		3.	- Shelf life after opening changed to 2 months
		4.1	- BA E-2212 Acylation Reagent now with white cap
		5.	- Sample collection and storage
		7.	- Calculation of results clarified
		7.1	- Typical standard curve updated
		9.1	- Lot-to-Lot and LOB/LOQ added
		9.2	- Metrological traceability added
		10.	- References updated
		11.	- Changes added
18.0-r	2024-02-15	4.1	- Hazard labelling updated according to SDS
		9.1	- Lot-to-Lot updated

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Use-by date	LOT	Batch code		
	Consult instructions for use	CONT	Content		
	Caution	REF	Catalogue number		Distributor
	Date of manufacture			RUO	For research use only!