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**Instructions for use**
Kynurenine ELISA

Please use only the valid version of the Instructions for use provided with the kit

REF**BA E-2200**

96

IVD

1. Introduction

1.1 Intended use and principle of the test

Enzyme Immunoassay for the quantitative determination of L-Kynurenine in serum and plasma samples.

After acylation Kynurenine 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 acylated standards, controls and samples and the solid phase bound analyte compete for a fixed number of antibody binding sites. When 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. 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.

1.2 Clinical application

Kynurenine is a non-proteinogenic amino acid that is produced as a metabolic intermediate during the degradation of tryptophan. The degradation of tryptophan is catalyzed by the inducible enzyme indolamine-2,3-dioxygenase (IDO). The product is kynurenine. Cytokines, in particular interferon- γ , influence the activity of the IDO, so that's why the kynurenine path is closely linked to the immune system. Kynurenine can be further converted to neuroprotective kynurenic acid, but also to neurotoxic quinolinic acid. Disorders of the tryptophan kynurenine metabolism are associated with different disease patterns, such as stress, cancer, and depression. The latter can be treated by tryptophan administration. This requires a determination of the kynurenine to tryptophan ratio, which is a reliable marker for the IDO activity. If no increased IDO activity is detected, the administered tryptophan can serve as the starting product for serotonin synthesis.

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 a certain type of sample 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 Stop Solution containing 0.25 M H₂SO₄. 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.

2.2.1 Interfering substances

Serum/Plasma

Samples containing precipitates or fibrin strands or which are haemolytic or lipemic might cause inaccurate results.

2.2.2 Drug interferences

There are no known substances (drugs) which ingestion interferes with the measurement of kynurenine level in the sample.

2.2.3 High-Dose-Hook effect








No hook effect was observed in this test.

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 Content of the kit

BA D-0032		Microtiter Plate - Ready to use
Content:	1 x 96 well plate, empty	
BA D-0090		Adhesive Foil - Ready to use
Content:	Adhesive Foils in a resealable pouch	
Volume:	1 x 4 foils	
BA E-0030		Wash Buffer Concentrate - Concentrated 50x
Content:	Buffer with a non-ionic detergent and physiological pH	
Volume:	1 x 20 ml/vial, light purple cap	
BA E-0040		Enzyme Conjugate - Ready to use
Content:	Goat anti-rabbit immunoglobulins conjugated with peroxidase	
Volume:	1 x 12 ml/vial, red cap	
BA E-0055		Substrate - Ready to use
Content:	Chromogenic substrate containing tetramethylbenzidine, substrate buffer and hydrogen peroxide	
Volume:	1 x 12 ml/vial, black cap	
BA E-0080		Stop Solution - Ready to use
Content:	0.25 M sulfuric acid	
Volume:	1 x 12 ml/vial, light grey cap	
Hazards identification:		
	H290 May be corrosive to metals. H314 Causes severe skin burns and eye damage.	

BA E-2231 **KYN** **Kynurenine Microtiter Strips** - Ready to use
 Content: 1 x 96 well (12x8) antigen precoated microwell plate in a resealable pouch with desiccant

BA E-2210 **AS/KYN** **Kynurenine Antiserum** - Ready to use
 Content: Rabbit anti-kynurenine antibody, blue coloured
 Volume: 1 x 6 ml/vial, blue cap

Standards and Controls - Ready to use

Cat. no.	Component	Colour/Cap	Concentration ng/ml	Concentration nmol/l	Volume/Vial
BA E-2201	STANDARD A	white	0	0	4 ml
BA E-2202	STANDARD B	light yellow	100	480	4 ml
BA E-2203	STANDARD C	orange	300	1 440	4 ml
BA E-2204	STANDARD D	dark blue	1 000	4 800	4 ml
BA E-2205	STANDARD E	light grey	3 000	14 400	4 ml
BA E-2206	STANDARD F	black	10 000	48 000	4 ml
BA E-2251	CONTROL 1	light green	Refer to QC-Report for expected value and acceptable range!		4 ml
BA E-2252	CONTROL 2	dark red			4 ml

Conversion: Kynurenine (ng/ml) x 4.80 = Kynurenine (nmol/l)

Content: TRIS buffer with non-mercury stabilizer, spiked with defined quantity of kynurenine

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, green cap

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

- Calibrated precision pipettes to dispense volumes between 20 – 500 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
- Temperature controlled incubator (37 °C) or similar heating device
- Microtiter plate shaker (shaking amplitude 3 mm; approx. 600 rpm)
- Absorbent material (paper towel)
- Water (deionized, distilled, or ultra-pure)
- Vortex mixer

5. Sample collection and storage

Plasma

Whole blood should be collected into centrifuge tubes containing EDTA as anti-coagulant (Monovette™ or Vacuette™) and centrifuged according to manufacturer's instructions immediately after collection.

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.

Serum

Collect blood by venipuncture (Monovette™ or Vacuette™ 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 absorption values may vary if a thermostat is not used. 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.

6.1 Preparation of reagents

Wash Buffer

Dilute the 20 ml Wash Buffer Concentrate with water (deionized, distilled, or ultra-pure) to a final volume of 1000 ml.

Storage: 1 month 2 – 8 °C.

Acylation Reagent

The Acylation Reagent has a freezing point of 18.5 °C. To ensure that the Acylation Reagent forms a homogeneous, 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 Acylation

1.	Pipette 10 µl of the standards, controls and samples into the appropriate wells of the Microtiter Plate .
2.	Add 250 µl of the Acylation Buffer to all wells.
3.	Add 25 µl of the Acylation Reagent to all wells and mix shortly.
4.	Cover the plate with Adhesive Foil and incubate 90 min at 37 °C .
5.	Use 20 µl for the ELISA!

6.3 Kynurenine ELISA

1.	Pipette 20 µl of the prepared standards, controls and samples into the appropriate wells of the Kynurenine Microtiter Strips .
2.	Pipette 50 µl of the Kynurenine Antiserum into all wells and mix shortly.
3.	Cover 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 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. 600 rpm).
7.	Discard or aspirate the contents of the wells. Wash the plate 4 x by adding 300 µl of 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. 600 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).

7. Calculation of results

Measuring range	Kynurenine
	63.3 – 10 000 ng/ml

The standard 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 and have to be reported as being positive.

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

Conversion

$$\text{Kynurenine (ng/ml)} \times 4.80 = \text{Kynurenine (nmol/l)}$$

Expected reference values

It is strongly recommended that each laboratory should determine its own reference value.

Plasma / Serum
237.4 – 754.2 ng/ml

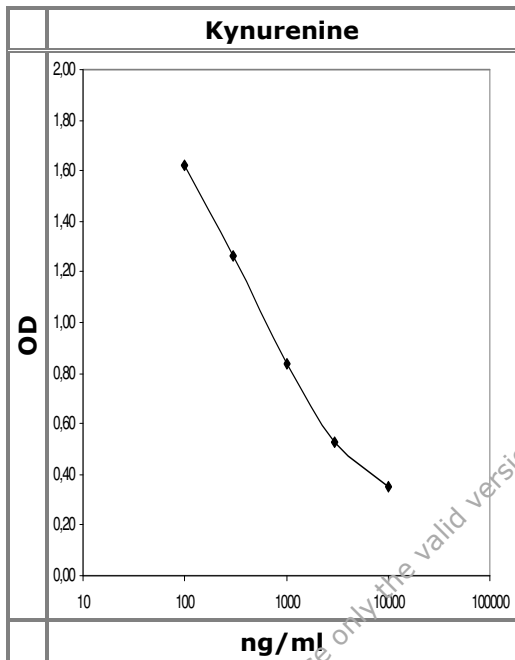
7.1 Quality control

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)	Kynurenine
	45.7 ng/ml

Analytical Specificity (Cross Reactivity)	Substance	Cross Reactivity (%)
	L-Kynurenine	100
	5-Hydroxy-DL-Tryptophan, Tyrosin, Phenylalanin, Serotonin, L-Asparagin, Kynurenic Acid	0.05
	Tryptophan	0.25
	3-Hydroxy-DL-Kynurenin	0.36

Precision Serum							
Intra-Assay				Inter-Assay			
Sample	(ng/ml)	SD	CV (%)	Sample	(ng/ml)	SD	CV (%)
1 (n = 20)	382.3	49.4	12.9	1 (n = 20)	373.8	65	17.5
2 (n = 20)	963.0	99.3	10.3	2 (n = 20)	893.5	119	13.3
3 (n = 20)	2242.0	244.8	10.9	3 (n = 20)	2050.6	295	14.4

Precision Plasma							
Intra-Assay				Inter-Assay			
Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)	Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
1 (n = 20)	386.5	57.1	14.8	1 (n = 20)	353.7	43	12.3
2 (n = 20)	986.9	90.4	9.2	2 (n = 20)	870.1	62	7.1
3 (n = 20)	2383.8	278.4	11.7	3 (n = 20)	1918.1	164	8.5
Linearity		Range Linearity %		Mean Linearity %		Serial dilution up to	
	Serum	90 - 104		95		1:128	
	Plasma	89 - 102		94		1:128	

Recovery	Serum	Range Recovery (%)	Mean Recovery (%)	% Recovery after spiking
	Sample 1	90 - 109	101	
	Sample 2	90 - 96	93	
	Sample 3	95 - 118	109	
	Plasma	Range Recovery (%)	Mean Recovery (%)	% Recovery after spiking
	Sample 1	82 - 106	96	
	Sample 2	90 - 104	99	
	Sample 3	97 - 110	103	







Method Comparison: ELISA vs. LC-MS/MS	Plasma	LC-MS/MS = 0.9x+71.5	R ² =0.9355; N = 30
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9. References/Literature

- (1) Lapin et al. Intensification of the central serotonergic processes as a possible determinant of the thymoleptic effect. *Lancet*, 1(7586):132-6 (1969)
- (2) Milton et al. Relationship between interferon-gamma, indoleamine 2,3-dioxygenase, and tryptophan catabolism. *FASEB J.*, 5(11): 2516-22 (1991)
- (3) Oxenkrug et al. Tryptophan kynurenine metabolism as a common mediator of genetic and environmental impacts in major depressive disorder: the serotonin hypothesis revisited 40 years later. *Isr J Psychiatry Relat Sci.*, 47(1): 56-63 (2010)
- (4) W.H. de Jong et al. Plasma tryptophan, kynurenine and 3-hydroxykynurenine measurement using automated on-line solid-phase extraction HPLC-tandem mass spectrometry. *J. Chromatogr., B* 877: 603-609 (2009)

⚠ **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	IVD	For in-vitro diagnostic use only!
	Consult instructions for use	CONT	Content	CE	CE labelled
	Caution	REF	Catalogue number	RUO	For research use only!