IMMUNOASSAYS AND SERVICES
BIOGENIC AMINES & NEUROSCIENCE | ENDOCRINOLOGY | FOOD SAFETY

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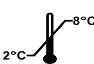
Instructions for use

Kynurenine ELISA

BA E-2200R



For research use only – Not for use in diagnostic procedures

1. Introduction

1.1 Intended use and principle of the test

Enzyme Immunoassay for the quantitative determination of L-Kynurenine in serum, plasma and various biological 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 Background

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.

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 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) 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 might cause inaccurate results. Hemolytic samples (up to 4 mg/ml hemoglobin), icteric samples (up to 50 mg/dl bilirubin) and lipemic samples (up to 1700 mg/dl triglycerides) have no influence on the assay 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 | 96 | **Microtiter Plate** - Ready to use

Content: 1 x 96 well plate, empty

BA D-0090 | FOILS | **Adhesive Foil** - Ready to use

Content: Adhesive Foils in a resealable pouch

Volume: 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, light 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

BA E-0055 | SUBSTRATE | **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-SOLN | **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.

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 instruction immediately after collection.
Haemolytic, icetric 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 instruction. Do not centrifuge before complete clotting has occurred.
Haemolytic, iceteric 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.

In case of overflow, read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to 405 nm.

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

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

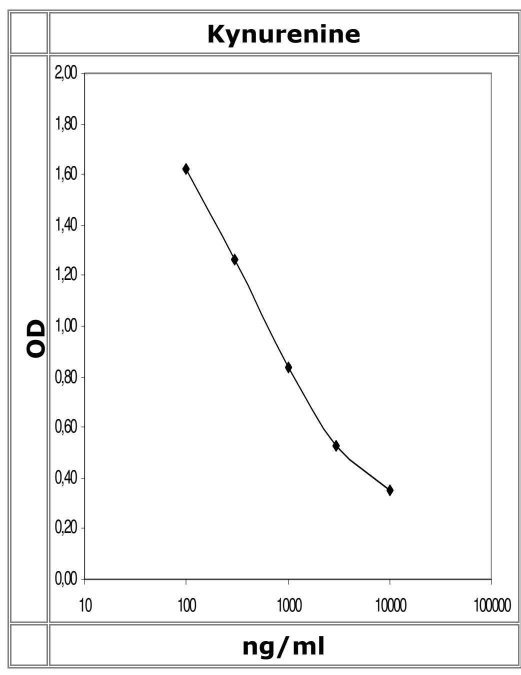
Plasma / Serum
237.4 – 754.2 ng/ml

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

	Substance	Cross Reactivity (%)
Analytical Specificity (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

9. References/Literature

(1) Lapin et al. Intensification of the central serotoninergic 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:

Symbol	Meaning	Symbol	Meaning	Symbol	Meaning
+2 / +8 °C	Storage temperature		Manufacturer	Σ	Contains sufficient for <n> tests
	Expiry date	LOT	Batch code		
	Consult instructions for use	CONT	Content		
	Caution	REF	Catalogue number	RUO	For research use only!