Instructions for Use

L-Kynurenin ELISA

IVD

CE

REF **EIA-5772**

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Please use only the valid version of the Instructions for Use provided with the kit.
Verwenden Sie nur die jeweils gültige, im Testkit enthaltene, Gebrauchsanweisung.
Si prega di usare la versione valida delle istruzioni per l'uso a disposizione con il kit.
Por favor, se usa solo la version valida de la metodico técnico incluido aqui en el kit.
Utilisez seulement la version valide des Instructions d'utilisation fournies avec le kit.

Table of Contents

1 INTENDED USE

This assay is intended for the quantitative determination of L-kynurenine in human EDTA plasma, serum and urine.

For *in vitro* diagnostic use only.

2 INTRODUCTION

L-kynurenine is the main product of the degradation of L-tryptophan, catalyzed by Indoleamine 2,3-dioxygenase (IDO).

L-kynurenine plays a key role as an immune suppressor in the course of infectious diseases (HIV[1], tuberculosis[2], borreliosis[3] etc.) and malignant diseases (colon cancer[4], lung cancer[5], [6], leukemia[7], Hodgkin lymphoma[8], cervical cancer[9]), where high kynurenine levels indicate a poor prognosis. Thus, L-kynurenine serves as a prognostic marker to predict the progression and the severity of the disease.

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8) Choe J et al. (2014) Indoleamine 2,3-dioxygenase (IDO) is frequently expressed in stromal cells of Hodgkin lymphoma and is associated with adverse clinical features : a retrospective cohort study, BMC Cancer 14(1), 1-9
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3 MATERIAL SUPPLIED

Label	Kit Components	Quantity
PLATE	Microtiter plate, pre-coated	12 x 8 wells
STD	Standards, ready-to-use (0, 0.1, 0.3, 1, 3, 10 µmol/L)	6 x 200 µL
CTRL 1	Control, ready-to-use (see specification for range)	1 x 200 µL
CTRL 2	Control, ready-to-use (see specification for range)	1 x 200 µL
WASHBUF A	Wash buffer concentrate, 10x	2 x 100 mL
AB	L-kynurenine antibody, lyophilized	1 x 1 vial
CONJ	Conjugate concentrate, peroxidase-labelled	1 x 65 µL
CONJBUF	Conjugate stabilizing buffer, ready-to-use	1 x 13 mL
REABUF	Reaction buffer, ready-to-use	1 x 110 mL
DER	Derivatization reagent, lyophilized	4 x 25 mg
DMSO	Dimethylsulfoxide (DMSO)	1 x 7 mL
SUB	Substrate (tetramethylbenzidine), ready-to-use	1 x 15 mL
STOP	Stop solution, ready-to-use	1 x 15 mL

4 MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultrapure water*
- Calibrated precision pipets and 10 µL - 1000 µL tips
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- Multi-channel pipets or repeater pipets
- Vortex
- Centrifuge, 3000 *g*
- Standard laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 7)

* DRG recommends the use of ultrapure water (Water Type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25 °C (≥18.2 MΩ cm).

5 STORAGE AND PREPARATION OF REAGENTS

- To run the assay more than once, ensure that reagents are stored at the conditions stated on the label. **Prepare only the appropriate amount necessary for each assay**. The kit can be used up to 4 times within the expiry date stated on the label.
- Reagents with a volume less than **100 µL** should be centrifuged before use to avoid loss of volume.
- **Preparation of the wash buffer**:
  The **wash buffer concentrate (WASHBUF A)** has to be diluted with ultrapure water **1:10** before use (100 mL WASHBUF A + 900 mL ultrapure water), mix well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals have to be redissolved at room temperature or in a water bath at 37 °C.
  The **WASHBUF A** is stable at **2 °C - 8 °C** until the expiry date stated on the label.
  Wash buffer (1:10 diluted WASHBUF A) can be stored in a closed flask at **2 °C - 8 °C for 1 month**.
- **DMSO** crystallizes at 2 °C - 8 °C. Before use, bring to room temperature to dissolve the crystals.
- Reconstitute the content of one vial of **derivatization reagent (DER)** (25 mg) **with 1.5 mL DMSO.** Allow to dissolve for 10 minutes and mix thoroughly with a vortex-mixer.
  The derivatization reagent must be **prepared immediately before use**. When more than one vial is to be used, combine the contents and mix prior to use. Discard any rest of the reagent after use.
  Please note: DMSO attacks all plastics but not polypropylene products and laboratory glass.
- The **lyophilized L-kynurenine antibody (AB)** is stable at **2 °C - 8 °C** until the expiry date stated on the label. Reconstitute the AB with **6 mL of wash buffer**.
  L-kynurenine antibody (reconstituted AB) **can be stored at 2 °C - 8 °C for 2 months.**
- **Preparation of the conjugate**:
  Before use, the conjugate concentrate has to be diluted **1:201** with **conjugate stabilizing buffer (CONJBUF)** (e.g. 60 µL CONJ + 12 mL CONJBUF, prepare only the required amount).
  The **CONJ** is stable at **2 °C - 8 °C** until the expiry date stated on the label.
  Conjugate (1:201 diluted CONJ) **can be stored at 2 °C - 8 °C for 1 month.**
- All other test reagents are ready-to-use.
  Test reagents are stable until the expiry date (see label) when stored at **2 °C - 8 °C.**

6 STORAGE AND PREPARATION OF SAMPLES

EDTA plasma, serum, urine

In the samples, L-kynurenine is stable for 72 h at 2 °C - 8 °C and at room temperature.

For longer storage keep samples frozen at -20 °C.

Samples are used **undiluted**.

For sample preparation, a derivatization reagent for derivatization of L-kynurenine is added (see sample preparation procedure).

7 ASSAY PROCEDURE

7.1 Principle of the test

This ELISA is designed for the quantitative determination of L-kynurenine. The assay is based on the method of competitive enzyme linked immunoassays.

The sample preparation includes the addition of a derivatization reagent for kynurenine derivatization. Afterwards, the treated samples and a polyclonal L-kynurenine-antiserum are incubated in the wells of a microtiter plate coated with L-kynurenine-derivative (tracer). During the incubation period, the target L-kynurenine in the sample competes with the tracer, immobilized on the wall of the microtiter wells, for the binding of the polyclonal antibodies.

During the second incubation step, a peroxidase-conjugated antibody is added to each microtiter well to detect the anti-kynurenine antibodies. After washing away the unbound components, tetramethylbenzidine (TMB) is added as a peroxidase substrate. Finally, the enzymatic reaction is terminated by an acidic stop solution. The color changes from blue to yellow, and the absorbance is measured in the photometer at 450 nm. The intensity of the yellow color is inverse proportional to the L-kynurenine concentration in the sample; this means, high L-kynurenine concentration in the sample reduces the concentration of tracer-bound antibodies and lowers the photometric signal. A dose response curve of absorbance unit (optical density, OD at 450 nm) vs. concentration is generated, using the values obtained from the standards. L-kynurenine, present in the patient samples, is determined directly from this curve.

7.2 Sample preparation procedure

Bring **all reagents and samples to room temperature** (15 °C - 30 °C) and mix well.

Derivatization of standards, controls and samples is carried out in single analysis in vials (e.g. 1.5 mL vials).

We recommend preparing one derivatization per standard, control and sample and transferring it in duplicate determinations into the wells of the microtiter plate.

1. Add **25 µL standard** (STD)/**control** (CTRL)/**sample** in the corresponding vials.
2. Add **1 mL reaction buffer** (REABUF) into each vial (STD, CTRL, sample).
3. Add **50 µL** of freshly prepared **derivatization reagent** into each vial (STD, CTRL, sample) and **mix thoroughly** by repeated inversion or several seconds on a vortex mixer. Incubate for **45 min at room temperature** (15 °C - 30 °C) on a **horizontal shaker**.

2 x 50 µL of the derivatized standards, controls and samples are used in the ELISA as duplicates.

7.3 Test procedure

Mark the positions of standards/controls/samples in duplicate on a protocol sheet.

Take as many microtiter strips (PLATE) as needed from the kit. Store unused strips covered at 2 °C - 8 °C. Strips are stable until expiry date stated on the label.

1. **Before use**, wash the wells **5 times** with **250 µL wash buffer**.
   After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
2. For the analysis in duplicate take **2 x 50 µL** of the **derivatized standards/controls/samples** out of the vials and add into the respective wells of the microtiter plate.
3. Add **50 µL L-kynurenine antibody** into each well.
4. Cover the strips tightly and incubate for **2 hours at room temperature** (15 °C - 30 °C) on a **horizontal shaker.**
5. Discard the content of each well and wash **5 times** with **250 µL wash buffer**.
   After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
6. Add **100 µL conjugate** into each well.
7. Cover the strips and incubate for **1 hour** at **room temperature** (15 °C - 30 °C) on a **horizontal shaker**.
8. Discard the content of each well and wash **5 times** with **250 µL wash buffer**.
   After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
9. Add **100 µL substrate** (SUB) into each well.
10. Incubate for **10-15 min*** **at room temperature** (15 °C - 30 °C) in the **dark.**
11. Add **100 µL stop solution** (STOP) into each well and mix well.
12. Determine **absorption immediately** with an ELISA reader at **450 nm** against 620 nm (or 690 nm) as a reference. If no reference wavelength is available, read only at 450 nm. If the extinction of the highest standard exceeds the range of the photometer, absorption must be measured immediately at **405 nm** against 620 nm (690 nm) as a reference.

* The intensity of the color change is temperature sensitive. We recommend observing the color change and stopping the reaction upon good differentiation.

For automated ELISA processors, the given protocol may need to be adjusted according to the specific features of the respective automated platform. For further details please contact your supplier or DRG.

8 RESULTS

The following algorithms can be used alternatively to calculate the results. We recommend using the 4 parameter algorithm.

1. **4 parameter algorithm**
   It is recommended to use a linear ordinate for optical density and a logarithmic abscissa for concentration. When using a logarithmic abscissa, the zero standard must be specified with a value less than 1 (e.g. 0.001).
2. **Point-to-point calculation**
   We recommend a linear ordinate for optical density and a linear abscissa for concentration.
3. **Spline algorithm**
   We recommend a linear ordinate for optical density and a linear abscissa for concentration.

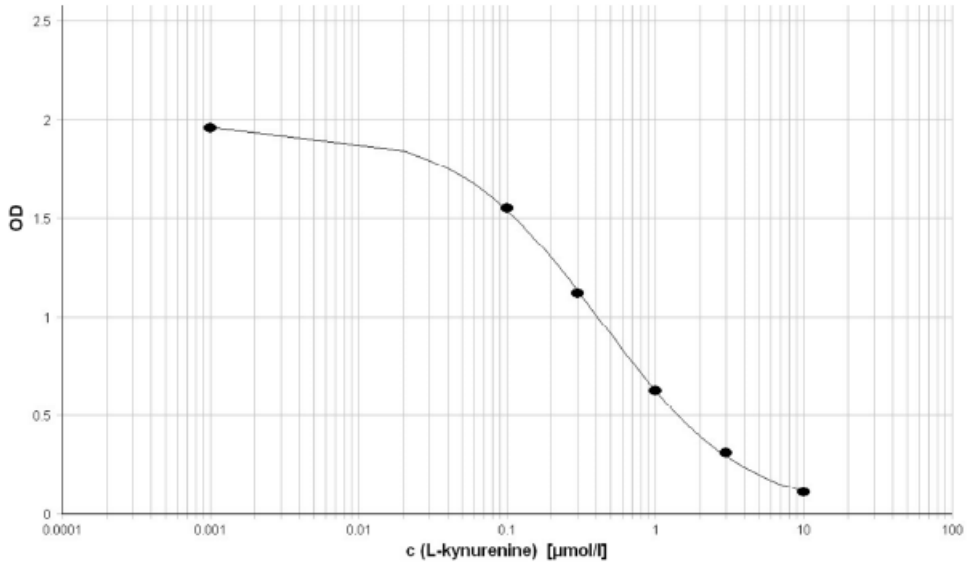
The plausibility of the pairs of values should be examined before the automatic evaluation of the results. If this option is not available with the used program, a control of the paired values should be done manually.

EDTA plasma, serum, urine

The concentrations can be determined directly from the standard curve in µmol/L. **No factor** is required.

In case another dilution factor has been used, multiply the obtained result by the dilution factor used.

In the following, an example of a calibration curve is given. Do not use it for the calculation of your results.



9 LIMITATIONS

Samples with concentrations above the measurement range (see definition below) must be diluted with reaction buffer and re-assayed. Please consider this dilution when calculating the results.

Samples with concentrations lower than the measurement range (see definition below) cannot be clearly quantified.

The upper limit of the measurement range can be calculated as:

highest concentration of the standard curve × sample dilution factor to be used

The lower limit of the measurement range can be calculated as:

LoB × sample dilution factor to be used

10 QUALITY CONTROL

DRG recommends the use of external controls for internal quality control, if possible.

Control samples should be analyzed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the patient samples may not be valid if within the same assay one or more values of the quality control samples are outside of the acceptable limits.

10.1 Reference Range

Based on internal studies with serum samples of apparently healthy persons a mean value of 1.80 µmol/L was estimated (n = 70). The standard deviation was 0.44 µmol/L.

From mean value ±2 × SD a normal range of 0.92 – 2.68 µmol/L was estimated.

We recommend each laboratory to establish its own reference range.

11 PERFORMANCE CHARACTERISTICS

11.1 Precision and reproducibility

Intra-Assay (n = 14)

Sample	L-kynurenine [µmol/L]	CV [%]
1	0.82	7.6
2	2.86	6.2

Inter-Assay (n = 8)

Sample	L-kynurenine [µmol/L]	CV [%]
1	0.80	9.2
2	2.80	6.2

11.2 Spiking recovery

Three samples were spiked with different L-kynurenine concentrations and measured in this assay (n = 2). The mean recovery rate was 102.5 %.

Sample	Spike [µmol/L]	L-kynurenine expected [µmol/L]	L-kynurenine measured [µmol/L]	Recovery [%]
A			2.48	
	1.5	3.98	4.49	112.8
	3.0	5.48	5.92	108.0
B			1.98	
	1.5	3.48	3.56	102.3
	3.0	4.98	4.81	96.6
C			2.03	
	1.5	3.53	3.45	97.7
	3.0	5.03	4.99	97.4

11.3 Dilution recovery

Two spiked samples were diluted and analyzed. The mean recovery rate was 100.3 % (n = 2).

Sample	Dilution	L-kynurenine expected [µmol/L]	L-kynurenine measured [µmol/L]	Recovery [%]
A			2.319	
	1:2	1.160	1.099	94.8
	1:3	0.773	0.748	96.8
	1:4	0.580	0.498	85.9
B			2.581	
	1:2	1.291	1.297	100.5
	1:3	0.860	0.877	101.9
	1:4	0.645	0.594	92.1
C			2.097	
	1:2	1.049	1.196	114.1
	1:3	0.699	0.822	117.6
	1:4	0.524	0.520	99.2

11.4 Analytical sensitivity

Limit of blank, LoB 0.076 µmol/L

Limit of detection, LoD 0.12 µmol/L

Limit of quantitation, LoQ 0.18 µmol/L

The evaluation was performed according to the CLSI guideline EP-17-A2. The specified accuracy goal for the LoQ was 15 % CV.

11.5 Specificity

The specificity of the antibody was tested by measuring the cross-reactivity against compounds with structural similarity to L-kynurenine. The specificity is calculated in percent in relation to the L-kynurenine binding activity.

3-HK (3-hydroxy-DL-kynurenine)	< 0.5 %
L-tryptophan	< 0.08 %
5-HTP (5-hydroxytryptophan)	< 0.01 %
Serotonin (5-HT, 5-hydroxytryptamine)	< 0.01 %
5-HIAA (5-hydroxyindoleacetic acid)	< 0.01 %
Quinolinic acid	< 0.01 %
Kynurenic acid	< 0.01 %
Picolinic acid	< 0.01 %

12 PRECAUTIONS

- All reagents in the kit package are for in vitro diagnostic use only.
- Human materials used in kit components were tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.
- Kit reagents contain sodium azide or ProClin as bactericides. Sodium azide and ProClin are toxic. Substrates for the enzymatic color reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes
- The stop solution consists of sulfuric acid, which is a strong acid. Although diluted, it still must be handled with care. It can cause burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped up immediately with copious quantities of water. Do not breathe vapour and avoid inhalation.

13 TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay. Furthermore, we recommend not assembling wells of different microtiter plates for analysis, even if they are of the same batch.
- Control Samples should be analyzed with each run.
- Reagents should not be used beyond the expiration date stated on the kit label.
- Substrate solution should remain colorless until use.
- To ensure accurate results proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- Do not mix plugs and caps from different reagents.
- The assay should always be performed according to the enclosed manual.

14 GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- The guidelines for medical laboratories should be followed.
- Incubation time, incubation temperature, and pipetting volumes of the different components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. DRG can therefore not be held responsible for any damage resulting from incorrect use.

15 REFERENCES / LITERATURE

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SYMBOLS USED

Symbol	English	Deutsch	Italiano	Español	Français
CE	European Conformity	CE-Konformitäts-kennzeichnung	Conformità europea	Conformidad europea	Conformité normes européennes
	Consult instructions for use *	Gebrauchsanweisung beachten *	Consultare le istruzioni per l'uso	Consulte las instrucciones de uso	Consulter les instructions d'utilisation
IVD	*In vitro* diagnostic medical device *	*In-vitro*-Diagnostikum *	Dispositivo medico-diagnostico in vitro	Producto sanitario para diagnóstico In vitro	Dispositif médical de diagnostic in vitro
REF	Catalogue number *	Artikelnummer *	Numero di Catalogo	Nûmero de catálogo	Référence de catalogue
LOT	Batch code *	Chargencode *	Codice del lotto	Codigo de lote	Numéro de lot
	Contains sufficient for <n> tests *	Ausreichend für <n> Prüfungen *	Contenuto sufficiente per "n" saggi	Contenido suficiente para <n> ensayos	Contenu suffisant pour "n" tests
	Temperature limit *	Temperaturbegrenzung *	Temperatura di conservazione	Temperatura de conservacion	Température de conservation
	Use-by date *	Verwendbar bis *	Utilizzare prima del	Establa hasta	Utiliser jusque
	Manufacturer *	Hersteller *	Fabbricante	Fabricante	Fabricant
	Caution *	Achtung *			
RUO	For research use only	Nur für Forschungszwecke	Solo a scopo di ricerca	Sólo para uso en investigación	Seulement dans le cadre de recherches
Distributed by	Distributed by	Vertreiber	Distributore	Distribuidor	Distributeur
Content	Content	Inhalt	Contenuto	Contenido	Contenu
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantità	Volumen/Número	Volume/Quantité