Human Kynurenine ELISA Kit

Enzyme Immunoassay for the quantification of Human Kynurenine in serum and plasma (EDTA).

Catalog number: ARG82779

Package: 96 wells

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INTRODUCTION

L-Kynurenine is a metabolite of the amino acid L-tryptophan used in the production of niacin.

Kynurenine is synthesized by the enzyme tryptophan dioxygenase, which is made primarily but not exclusively in the liver, and indoleamine 2,3-dioxygenase, which is made in many tissues in response to immune activation. Kynurenine and its further breakdown products carry out diverse biological functions, including dilating blood vessels during inflammation and regulating the immune response. Some cancers increase kynurenine production, which increases tumor growth.

Evidence suggests that increased kynurenine production may precipitate depressive symptoms associated with interferon treatment for hepatitis C. Cognitive deficits in schizophrenia are associated with imbalances in the enzymes that break down kynurenine. Kynurenine production is increased in Alzheimer's disease and cardiovascular disease where its metabolites are associated with cognitive deficits and depressive symptoms. Kynurenine is also associated with tics.

Kynureninase catabolizes the conversion of kynurenine into anthranilic acid while kynurenine-oxoglutarate transaminase catabolizes its conversion into kynurenic acid. Kynurenine 3-hydroxylase converts kynurenine to 3-hydroxykynurenine. [Provided by Wikipedia: Kynurenine]

PRINCIPLE OF THE ASSAY

This assay employs the competitive quantitative enzyme immunoassay technique. First, the target in serum or plasma are collection by acylated procedure. The antigen has been pre-coated onto a microtiter plate. Acylated Controls, Standards or samples and the solid phase bound analytes compete for a number of antibody binding sites. When the system is in equilibrium, free antigen and free antigen-antibody complexes are removed by washing procedure. Anti-rabbit IgG-peroxidase conjugate is added to each well and incubated. After washing away any unbound antibody conjugate, a substrate solution (TMB) is added to the wells and color develops in inversely proportion to the amount of kynurenine present in the samples. The color development is stopped by the addition of STOP solution and the intensity of the color is measured at a wavelength of 450 nm. The concentration of kynurenine in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store all other components at 2-8°C. Once opened the reagents are stable for 1 month when stored at 2-8°C. Use the kit before expiration date.

Component	Quantity	Storage information
Kynurenine Coated microplate	8 X 12 strips	4°C
Standards A to F (0, 100, 300, 1000, 3000, 10000 ng/mL)	4 mL each (ready to use)	4°C
Control 1 (Accept conc.: 400 ng/ml ± 40%)	4 ml (ready to use)	4°C
Control 2 (Accept conc.:2000 ng/ml ± 40%)	4 ml (ready to use)	4°C
Kynurenine Antiserum	6 ml (ready to use)	4°C
Acylation Buffer	30 mL (ready to use)	4°C
Acylation Reagent	3 mL (ready to use)	4°C
Acylation Plate	1 X 96 wells (Ready-to-use)	4°C
Anti-rabbit IgG-peroxidase conjugate	12 mL (ready to use)	4°C
50X Wash Buffer	20 mL	4°C
TMB substrate	12 mL (ready to use)	4°C (protect from light)
STOP solution	12 mL (ready to use)	4°C
Adhesive Foil	4 pieces	RT

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: read at 620-650 nm as reference wavelength)
- Microplate shaker (shaking amplitude 3 mm; approx. 600 rpm)
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)
- Temperature controlled incubator (37°C) or similar heating device

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the unopened reagents at 2 -8° C until expiration date. 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.
- Remove the number of strips required and return unused strips to the pack and reseal.
- 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.
- If crystals are observed in the 50X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- 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.

- Ensure complete reconstitution and dilution of reagents prior to use.
- The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- 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.
- Change pipette tips between the addition of different reagent or samples.
- In rare cases residues of the blocking and stabilizing reagent can be seen in the wells of Serotonin coated microtiter strips as small, white dots or lines. These residues do not influence the quality of the product.

SAMPLE COLLECTION & STORAGE INFORMATION

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes.

Plasma: Plasma: Collect blood with EDTA, heparin or citrate and centrifuge at 2000 x g and 4°C for 10 minutes. Collect the plasma layer and store on ice.

Note:

1. Avoid disturbing the white buffy layer when collection serum/plasma sample.
2. Do not use haemolytic, icteric or lipaemic specimens.
3. Samples containing sodium azide should not be used in the assay.
4. Specimens should be capped and may be stored for up to 48 hours at 2-8°C prior to assaying. Specimens stored for a longer time (up to 3 months) should be frozen only once at-20°C prior to assay. Thawed samples should be inverted several times prior to testing.

REAGENT PREPARATION

- 1X Wash Buffer: Dilute 50X Wash Buffer into distilled water to yield 1X Wash Buffer. (E.g., add 20 mL of 50X Wash Buffer into 980 mL of distilled water to a final volume of 1000 mL) The 1X Wash Buffer is stable for up to 4 weeks at 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.

ASSAY PROCEDURE

Acylation

1. Add **10 µL** of the **Standards**, **Controls** and **samples** into the appropriate wells of the Acylation Plate.
2. Add **250 µL** of the **Acylation Buffer** to all wells.
3. Add **25 µL** of the Acylation Reagent to all wells and incubate 1 min at RT (20 – 25 °C) on a microplate shaker at approx. 600 rpm.
4. Cover the plate with Adhesive Foil and incubate at **37°C** for **90 minutes**
5. Use **20 µL** for the ELISA

Kynurenine ELISA

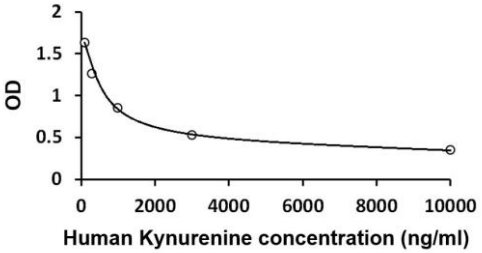
All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Standards and samples should be assayed in duplicates.

1. Add **20 µL** of **prepared Standards, Controls and samples** into the appropriate wells of the Kynurenine Coated Microplate.
2. Add **50 µL** of **Kynurenine Antiserum** into all wells and mix well.

3. Cover the plate with Adhesive Foil and incubate at **2-8°C** for **15-20 hours.**
4. Aspirate each well and wash, repeating the process 3 times for a **total 4 washes**. Wash by filling each well with **1× Wash Buffer** (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add **100 µL** of **Anti-rabbit IgG-peroxidase conjugate** into all wells.
6. Incubate at **RT** for **30 minutes** on a microplate shaker at approx. 600 rpm.
7. Aspirate each well and **wash as step 4.**
8. Add **100 µL** of **TMB Substrate** to all wells, including the blank wells. Incubate in the dark for **20-30 minutes** at **RT** on a microplate shaker at approx. 600 rpm.
9. Add **100 µL** of **Stop Solution** to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution. The color of the solution should change from blue to yellow.
10. Read the OD with a microplate reader at **450 nm** immediately. (optional: read at 620 nm as reference wavelength) It is recommended reading the absorbance **within 10 minutes** after adding the stop solution.

EXAMPLE OF TYPICAL STANDARD VALUES

The following figures demonstrate typical results with the Human Kynurenine ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of Controls, standards and samples.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
4. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

5. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.
6. Conversion: Kynurenine (ng/ml) x 4.80 = Kynurenine (nmol/l)
7. Expected reference value:

Plasma / Serum
237.4 – 754.2 ng/mL 1.1 – 3.6 nmol/ml

QUALITY ASSURANCE

Sensitivity

The sensitivity of the Human Kynurenine ELISA kit is 45.7 ng/mL.

Specificity

Substance	Cross Reactivity (%)
L-Kynurenine	100
5-Hydroxy-DL-Tryptophan, Tyrosin, Phenylalanin, Serotonin, L-Asparagin, Kynurenic Acid	0.05
Tryptophan	0.18
3-Hydroxy-DL-Kynurenin	0.3

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 11-15.5% and CV value of inter-assay precision was 7.1-17.7%.

Recovery

Serum: 93-109%; Plasma: 96-103%