Tryptophan ELISA Kit

Catalog Number KA1916

96 assays

Version: 08

Intended for research use only

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Introduction

Intended Use

Enzyme Immunoassay for the quantitative determination of Tryptophan in urine, serum, and plasma samples.

Background

L-Tryptophan is one of the essential amino acids for the human metabolism and must be part of its diet.
In humans it serves as precursor for the synthesis of the neurotransmitters serotonin and tryptamine as well as for the synthesis of nicotinic acid and the epiphyseal hormone melatonin. Tryptophan is catabolized to kynurenine through the enzyme IDO (indoleamine-2,3-dioxygenase). Increased IDO activity is an expression of neuro-endocrine-immunological dysregulation, which is often associated with depressive symptoms such as bipolar disorder (manic depression). In addition Tryptophan and its metabolites regulate neurobehavioral effects such as appetite, sleeping-waking-rhythm and pain perception.

Principle of the Assay

After extraction and derivatization Tryptophan 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 derivatized 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 standard curve prepared with known standards.

General Information

Materials Supplied

List of component

Component	Description	Amount
Adhesive Foil	Ready to use. Adhesive Foils in a resealable pouch.	4 slices
Reaction Plate	Ready to use. 1 x 96 well plate, empty in a resealable pouch.	1 plate
Wash Buffer Concentrate (50x)	Concentrated 50x. Buffer with a non-ionic detergent and physiological pH.	20 mL
Enzyme Conjugate	Ready to use, goat anti-rabbit immunoglobulins conjugated with peroxidase.	12 mL
Substrate	Ready to use, chromogenic substrate containing tetramethylbenzidine, substrate buffer and hydrogen peroxide.	12 mL
Stop Solution	Ready to use, 0.25 M sulfuric acid. Hazards identification:  H290 May be corrosive to metals.	12 mL
Tryptophan Microtiter Strips	Ready to use, antigen precoated microwell plate in a resealable pouch with desiccant.	96 (8x12) wells
Tryptophan Antiserum	Ready to use, rabbit anti-tryptophan antibody, blue coloured.	6 mL
Assay Buffer	Ready to use, buffer with alkaline pH.	20 mL
Equalizing Reagent	Lyophilized protein.	1 vial
Q-Buffer	Ready to use. TRIS buffer.	20 mL
PBS	Ready to use. Phosphate Buffered Saline.	20 mL
D-Reagent	Ready to use, crosslinking agent in dimethylsulfoxide. Hazards identification:  H317 May cause an allergic skin reaction.	4 mL
Precipitating Reagent	Ready to use, acidic reagent for precipitation of plasma/serum proteins, red coloured.	4 mL

Standards and Controls - Ready to use

Component	Concentration (µg/mL)	Concentration (µmol/L)	Volume
Standard A	0	0	4 mL
Standard B	2.5	12.2	4 mL
Standard C	7.5	36.7	4 mL
Standard D	25	122	4 mL
Standard E	75	367	4 mL
Standard F	250	1224	4 mL
Control 1	Refer to QC-Report for expected value and acceptable range.		4 mL
Control 2	Refer to QC-Report for expected value and acceptable range.		4 mL

*Conversion: Tryptophan (µg/mL) x 4.89 = Tryptophan (µmol/L)

Contents: Acidic buffer with non-mercury stabilized, spiked with defined quantity of tryptophan.

Storage Instruction

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.

Materials Required but Not Supplied

- ✓ Calibrated precision pipettes to dispense volumes between 10 – 300 µL; 12.5 mL
- ✓ Polystyrene or polypropylene tubes and suitable rack
- ✓ 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)
- ✓ Absorbent material (paper towel)
- ✓ Water (deionized, distilled or ultra-pure)
- ✓ Vortex mixer
- ✓ Centrifuge

Precautions for Use

- ✓ Procedural cautions, guidelines and warnings
  1. This kit is intended for research 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. The principles of Good Laboratory Practice (GLP) have to 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. Avoid repeated freezing and thawing of reagents and specimens.
5. For dilution or reconstitution purposes, use deionized, distilled or ultra-pure water.
6. 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.
7. Duplicate determination of sample is highly recommended to be able to identify potential pipetting errors.
8. 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.
9. Incubation times do influence the results. All wells should be handled in the same order and time intervals.
10. To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
11. A standard curve must be established for each run.
12. The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report.
13. 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.
14. 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.
15. 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.
16. For information on hazardous substances included in the kit please refer to Material Safety Data Sheet (MSDS). The Material Safety Data Sheet for this product is made available directly on the website of the manufacturer or upon request.
17. Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.
18. In case of any severe damage to the test kit or components, we have to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the office regulations.

✓ Limitations

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

✓ Interfering substances

- Serum/Plasma

  Samples containing precipitates or fibrin strands might cause inaccurate results.

  Hemolytic samples (up to 2 mg/mL hemoglobin), icteric samples (up to 50 mg/dL bilirubin) and lipemic samples (up to 1600 mg/dL triglycerides) have no influence on the assay results.

- 24-hour urine

  Please note the sample preparation! If the percentage of the final concentration of acid is too high, this will lead to incorrect results for the urine samples.

✓ Drug interferences

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

✓ High-Dose-Hook effect

No hook effect was observed in this test.

Assay Protocol

Reagent Preparation

- ✓ Wash Buffer

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

  Storage: 1 month at 2-8°C.
- ✓ Equalizing Reagent

  Reconstitute the Equalizing Reagent with 12.5 mL of Assay Buffer.

  Reconstituted Equalizing Reagent which is not used immediately has to be stored in aliquots for max 1 month at -20°C and may be thawed only once.
- ✓ D-Reagent

  The D-Reagent has a freezing point of 18.5°C. It must be ensured that the D-Reagent has reached room temperature and forms a homogeneous, crystal-free solution.
- ✓ Tryptophan 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.

Sample Preparation

- ✓ Sample collection and storage
- Plasma

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

  Fasting specimens or pre-feed specimens for children (2 - 3 hours after last meal) are advised.

  Haemolytic, 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 month) at -20°C.

  Repeated freezing and thawing should be avoided.
- Serum

  Collect blood by veinpuncture (Monovette™ or Vacuette™ for serum), allow to clot, and separate serum by centrifugation according to manufacturer's instructions at room temperature. Do not centrifuge before complete clotting has occurred.

  Fasting specimens or pre-feed specimens for children (2 - 3 hours after last meal) are advised.

  Haemolytic, 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 month) at -20°C.

  Repeated freezing and thawing should be avoided.

- Urine

  Spontaneous urine or 24-hour urine, collected in a bottle containing 10 - 15 mL of 6 M HCl, can be used.
  If 24-hour urine is used please record the total volume of the collected urine.
  Storage: up to 48 hours at 2-8°C, for longer periods (up to 6 month) at -20°C.
  Repeated freezing and thawing should be avoided. Avoid exposure to direct sunlight.

Assay 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 absorbance values may vary if a thermostat is not used. The higher the temperature, the higher the extinction values will be. Corresponding variations also apply to the incubation times. The optimal temperature during the Enzyme Immunoassay is between 20 – 25°C.

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

✓ Precipitation

1. Pipette 20 µL of standards, controls, and samples into the respective tubes.
2. Add 200 µL PBS to all tubes.
3. Add 25 µL Precipitating Reagent to all tubes.
4. Mix the tubes thoroughly (vortex) and centrifuge for 15 minutes at 3,000 x g.
5. Take 25 µL of the clear supernatant for the derivatization.

✓ Derivatization

1. Pipette 25 µL of the precipitated standards, controls and samples into the appropriate wells of the Reaction Plate.
2. Pipette 50 µL of the Equalizing Reagent into all wells.
3. Pipette 10 µL of the D-Reagent into all wells.
4. Cover plate with Adhesive Foil and incubate for 2 hours at RT (20-25°C) on a shaker (approx. 600 rpm).
5. Pipette 100 µL of the Q-Buffer into all wells.
6. Incubate for 10 min at RT (20-25°C) on a shaker (approx. 600 rpm).
7. Use 25 µL for the ELISA.

✓ Tryptophan ELISA

1. Pipette 25 µL of the prepared standards, controls and samples into the appropriate wells of the Tryptophan Microtiter Strips.
2. Pipette 50 µL of the Tryptophan Antiserum into all wells and mix shortly.

3. Cover plate with Adhesive Foil and incubate for 15-20 hours (overnight) at 2-8°C.
4. Remove the foil. Discard or aspirate the contents of the wells. Wash the plate 3 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 content of the wells. Wash the plate 3 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).

Data Analysis

Calculation of Results

✓ Measuring range:
Tryptophan: 0.73 - 250 µg/mL.

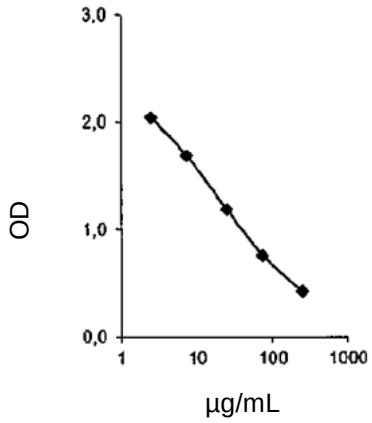
The calibration curve is obtained by plotting the absorbance readings (calculate the mean absorbance) of the standards (linear, y-axis) against the corresponding standard concentrations (logarithmic, x-axis).
Use non-linear regression for curve fitting (e.g. spline, 4- parameter, akima).
Note: This assay 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.
The total amount of Tryptophan excreted in urine during 24 h is calculated as following:
µg/24h = µg/L x L/24h

✓ Conversion
Tryptophan (µg/mL) x 4.89 = Tryptophan (µmol/L)

✓ Expected reference values
It is strongly recommended that each laboratory should determine its own reference values.
- Plasma/Serum: 9.3-17.0 µg/mL
- Spontaneous urine: 15.6-101 µmol/g creatinine

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

✓ Typical standard curve



Note: Example, do not use for calculation!

Performance Characteristics

✓ Analytical Sensitivity

	Tryptophan
LOB	0.48 ug/mL
LOD	0.65 ug/mL
LOQ	0.73 ug/mL

✓ Analytical Specificity (Cross Reactivity)

Substance	Cross Reactivity (%)
Tryptophan	100
5-Hydroxy-L-tryptophan	<0.01
Tryptamine	<0.01
5-Methoxy-L-tryptophan	<0.01
5-Hydroxytryptamine	<0.01
5-Methoxytryptophan	<0.01

✓ Precision

Intra-Assay			Inter-Assay		
Sample	Range (µg/mL)	CV (%)	Sample	Range (µg/mL)	CV (%)
1	3.3 ± 0.9	27	1	2.8 ± 0.5	17
2	7.3 ± 1.1	15	2	7.7± 1.1	14
3	23.2 ± 2.2	9	3	23.4 ± 3.4	15
4	67.6 ± 4.4	6	4	66.4 ± 7.5	11

✓ Linearity

Range (µg/mL)	Serial dilution up to	Range (%)
3.6-4.8	1:64	73-115

✓ Recovery

	Range (µg/mL)	Mean (%)	Range (%)
Urine	5.4-207	107	100-114
Serum	14.9-196	96	87-108
Plasma	12.1-202	100	89-110

✓ Method comparison versus LC-MS/MS

LC-MS/MS= 1.06 ELSA-2.9	r=0.99	n=41

Resources

References

1. El-Bakly et al. Hypericum Perforatum Decreased Hippocampus TNF-α and Corticosterone Levels with NoEffect on Kynurenine/Tryptophan Ratio in Bilateral Ovariectomized Rats. Korean J Physiol Pharmacol, 18: 133-139 (2014).
2. Nowak et al. Tryptophan hydroxylase-1 regulates immune tolearance and inflammation. The Journal of Experimental Medicine, 209 (11): 2127-2135 (2012).
3. Sorensen et al. Indoleamine 2,3-dioxygenasespecific, cytotoxic T cells as immune regulators. Blood, 117(7): 2200-2210 (2011)

Plate Layout

12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H