Tryptophan ELISA

Immunoassay for the quantitative determination of Tryptophan in urine, serum and plasma (EDTA) samples.

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 17-TRPHU-E01

Size: 96 wells

Version: 14.0 2020-03-26 - ALPCO 3.0

1. Introduction

1.1. Intended use

The Tryptophan ELISA is an Enzyme Immunoassay for the quantitative determination of Tryptophan in urine, serum, and plasma (EDTA) samples. For research use only. Not for use in diagnostic procedures.

1.2. 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.

1.3. 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). Research demonstrates that 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, research shows that tryptophan and its metabolites regulate neurobehavioral effects such as appetite, sleeping-waking-rhythm and pain perception.

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 the protocol for successful use of this kit. Only the test instruction provided with the kit is valid and must be used to run the assay. Reliable performance will only be attained by strict and careful adherence to the instructions provided. This assay was validated for the sample types indicated in Intended Use Section. Any off-label use of this kit is the responsibility of the user and the manufacturer cannot be held liable.
2. The principles of Good Laboratory Practice (GLP) must be followed.
3. To reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves, and protective glasses where necessary.
4. All kit reagents and samples should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and samples.
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 sample determinations are highly recommended to identify potential pipetting errors.
8. Once started, all steps should be completed without interruption. Make sure that the required reagents, materials and devices are prepared and ready at the appropriate time.
9. Incubation times influence results. 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 is skin and mucosa irritant. 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 Safety Data Sheet (SDS). The Safety Data Sheet for this product is made available directly on ALPCO's website.
17. Treat kit reagents as hazardous waste and dispose of according to applicable 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. Hemolyzed 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.

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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.

2.2.2. Drug Interferences

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

2.2.3. High-Dose-Hook Effect

No hook effect was observed in this assay.

3. Storage and Stability

Store unopened reagents at 2 - 8 °C until expiration date. Do not use components beyond their expiration dates. 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. Contents of the Kit

BA D-0090 FOILS **Adhesive Foil** - Ready to use
Contents: Adhesive Foils in a resealable pouch
Volume: 1 x 4 foils

BA D-0024 REAC-PLATE **Reaction Plate** - Ready to use
Contents: 1 x 96 well plate, empty in a resealable pouch

BA E-0030 WASH-CONC 50x **Wash Buffer Concentrate -** Concentrated 50x
Contents: 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
Contents: Goat anti-rabbit immunoglobulins conjugated with peroxidase
Volume: 1 x 12 mL/vial, red cap

BA E-0055 SUBSTRATE **Substrate** - Ready to use
Contents: Chromogenic substrate containing tetramethylbenzidine, substrate buffer and hydrogen peroxide
Volume: 1 x 12 mL/black vial, black cap

BA E-0080 STOP-SOLN **Stop Solution** - Ready to use
Contents: 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-2731 TRYP **Tryptophan Microtiter Strips** - Ready to use
Contents: 1 x 96 well (12x8) antigen precoated microwell plate in a resealable pouch with desiccant

BA E-2710 AS TRYP **Tryptophan Antiserum** - Ready to use
Contents: Rabbit anti-tryptophan antibody, blue colored
Volume: 1 x 6 mL/vial, blue cap

Standards and **Controls** - Ready to use

Cat. no.	Component	Color/Cap	Concentration µg/mL	Concentration µmol/L	Volume/ Vial
BA E-2701	STANDARD A	white	0	0	4 mL
BA E-2702	STANDARD B	light yellow	2.5	12.2	4 mL
BA E-2703	STANDARD C	orange	7.5	36.7	4 mL
BA E-2704	STANDARD D	dark blue	25	122	4 mL
BA E-2705	STANDARD E	light grey	75	367	4 mL
BA E-2706	STANDARD F	black	250	1,224	4 mL
BA E-2751	CONTROL 1	light green	Refer to QC-Report for expected value and acceptable range!		4 mL
BA E-2752	CONTROL 2	dark red			4 mL

Conversion: Tryptophan (µg/mL) x 4.89 = Tryptophan (µmol/L)
Contents: Acidic buffer with non-mercury stabilizer, spiked with defined quantity of tryptophan

BA E-2413 ASSAY-BUFF **Assay Buffer** - Ready to use
Contents: Buffer with alkaline pH
Volume: 1 x 20 mL/vial, yellow cap

BA E-2428 EQUA-REAG **Equalizing Reagent** - Lyophilized
Contents: Lyophilized protein

Volume: 1 vial, brown cap

BA E-2446 D-REAGENT **D-Reagent** - Ready to use
Contents: Crosslinking agent in dimethyl sulfoxide
Volume: 1 x 4 mL/vial, white cap

BA E-2458 Q-BUFFER **Q-Buffer** - Ready to use
Contents: TRIS buffer
Volume: 1 x 20 mL/vial, white cap

BA E-2788 PBS **PBS** - Ready to use
Contents: Phosphate Buffered Saline
Volume: 1 x 20 mL/vial, orange cap

BA E-2721 PREC-REAG **Precipitating Reagent** - Ready to use
Contents: Acidic reagent for precipitation of plasma/serum proteins, red colored
Volume: 1 x 4 mL/vial, white cap

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

- 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
- Timer

5. Sample Collection and Storage

Plasma
Whole blood should be collected by venipuncture into centrifuge tubes containing EDTA as anti-coagulant and centrifuged according to manufacturer's instructions at room temperature immediately after collection.
Fasting sample or pre-feed samples from children (2 - 3 hours after last meal) are advised.
Hemolyzed, icteric, and lipemic samples should not be used for the assay.
Storage: Up to 48 hours at 2 - 8 °C, for longer periods (up to 6 months) at -20 °C.
Repeated freezing and thawing should be avoided.

Serum
Collect blood by venipuncture, allow to clot, and separate serum by centrifugation according to manufacturer's instructions at room temperature. Do not centrifuge before complete clotting has occurred. Subjects receiving anticoagulant therapy may require increased clotting time.
Fasting sample or pre-feed samples from children (2 - 3 hours after last meal) are advised.
Hemolyzed, icteric, and lipemic samples should not be used for the assay.
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.

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 months) at -20 °C. Repeated freezing and thawing should be avoided. Avoid exposure to direct sunlight.

6. 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.

Binding of the antisera and 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.

6.1. Preparation of the 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 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 must be stored in aliquots for a maximum of 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.

6.2. Precipitation

1.	Pipette **20 µL** of the **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**.
⚠	Take **25 µL** of the clear supernatant for the **derivatization**.

6.3. 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 **Room Temperature** (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 **Room Temperature** (20 – 25 °C) on a **shaker** (approx. 600 rpm).

7.	**Use 25 µL for the ELISA!**

6.4. 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 content of the wells. Wash the plate **3 times** by adding **300 µL** of **Wash Buffer, discarding** the contents 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 **Room Temperature** (20 – 25 °C) on a **shaker** (approx. 600 rpm).
7.	Discard or aspirate the content of the wells. Wash the plate **3 times** by adding **300 µL** of **Wash Buffer, discarding** the contents 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 **Room Temperature** (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 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	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).

⚠ 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 must 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 hours is calculated as following:

µg/24h = µg/L x L/24h

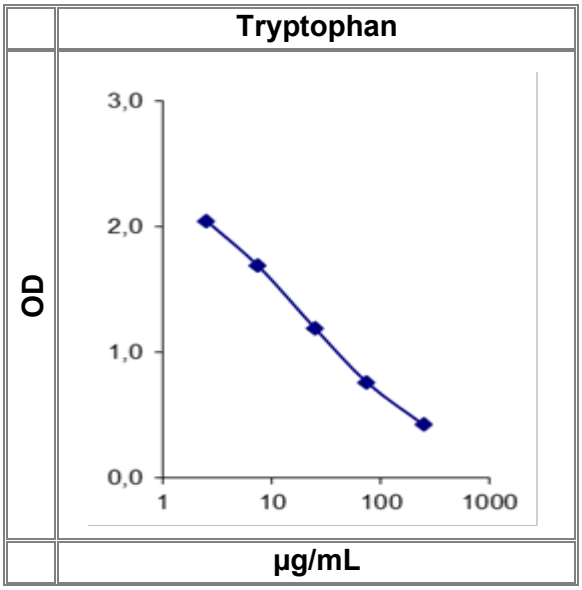
Conversion
Tryptophan (µg/mL) x 4.89 = Tryptophan (µmol/L)

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

		Tryptophan
Analytical Sensitivity	LOB	0.48 µg/ml
	LOD	0.65 µg/ml
	LOQ	0.73 µg/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-Methoxytryptamine	<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 – 14.8	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 ELISA – 2.9	r = 0.99	n = 41

References

1. El-Bakly et al. Hypericum Perforatum Decreased Hippocampus TNF-α and Corticosterone Levels with No Effect 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 tolerance and inflammation. The Journal of Experimental Medicine, 209(11): 2127-2135 (2012)
3. Sorensen et al. Indoleamine 2,3-dioxygenase specific, cytotoxic T cells as immune regulators. Blood, 117(7): 2200-2210 (2011)