Human Tryptophan ELISA Kit

Enzyme Immunoassay for the quantification of Tryptophan in serum, plasma (EDTA) and urine

Catalog number: ARG80483

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Tryptophan (symbol Trp or W) is a α-amino acid that is used in the biosynthesis of proteins. Many animals (including humans) cannot synthesize tryptophan: they need to obtain it through their diet, making it an essential amino acid. Tryptophan is among the less common amino acids found in proteins, but it plays important structural or functional roles whenever it occurs. For instance, tryptophan and tyrosine residues play special roles in "anchoring" membrane proteins within the cell membrane. In addition, tryptophan functions as a biochemical precursor for the following compounds:

- Serotonin (a neurotransmitter), synthesized by tryptophan hydroxylase.
- Melatonin (a neurohormone) is in turn synthesized from serotonin, via N-acetyltransferase and 5-hydroxyindole-O-methyltransferase enzymes.
- Niacin, also known as vitamin B3, is synthesized from tryptophan via kynurenine and quinolinic acids.
- Auxins (a class of phytohormones) are synthesized from tryptophan.

The disorder fructose malabsorption causes improper absorption of tryptophan in the intestine, reduced levels of tryptophan in the blood, and depression. [Provide by Wikipedia: Tryptophan]

PRINCIPLE OF THE ASSAY

The competitive ELISA uses the microtiter plate format. An antigen Tryptophan has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Tryptophan present compete for the fixed number of antibody binding site. After washing away any unbound substances, a Horseradish Peroxidase (HRP)-conjugated antibody specific for rabbit IgG is added to each well and incubate. The antibody bound to the solid phase is

detected by HRP-conjugated Ab 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.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	**Quantity**	**Storage information**
Reaction Plate	1 plate/96 wells	4°C.
Tryptophan-coated microtiter strips	8 X 12 strips	4°C.
Standard A-F (0, 2.5, 7.5, 25, 75, 250 µg/ml)	6 vials/4 ml	4°C, ready for use
Control 1 (10 µg/ml ± 40%)	4 ml	4°C, ready for use
Control 2 (30 µg/ml ± 40%)	4 ml	4°C, ready for use
Equalizing Reagent	1 vial	Lyophilized
D-Reagent	4 ml	4°C, ready for use
Q-Buffer	20 ml	4°C, ready for use
Enzyme-conjugated Antibody	12 ml	4°C, ready for use
Tryptophan antiserum	6 ml	4°C, ready for use
Assay buffer	20 ml	4°C, ready for use
50X Wash buffer	20 ml	4°C
Precipitating Reagent	4 ml	4°C, ready for use
PBS	20 ml	4°C, ready for use
TMB substrate	12 ml	4°C (Protect from light)
STOP solution	12 ml	4°C
Plate sealer	4 strips	RT

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Microplate shaker (~600 rpm)
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- 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.
- Briefly spin down the all components before use.
- If crystals are observed in the 50X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Precipitation may occur in 30X HRP-labeled antibody, however, there is no problem in the performance.
- Standard is lyophilized products. Be careful to open this vial.
- Use the Wash Buffer contained in this kit only. Insufficient washing may lead to the failure in measurement.
- Remove the Wash Buffer completely by tapping the microtiter plate on paper towel. Do not wipe wells with paper towel.

- Ensure complete reconstitution and dilution of reagents prior to use.
- Allow all reagents and samples to reach room temperature (20 – 25°C) and mix thoroughly by gentle inversion before use.
- 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.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Urine – Spontaneous or 24-hour urine, collected in a bottle containing 10-15 ml of 6 M HCL, should be used. Store samples at ≤ -20 °C for up to 6 month. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C for up to 6 month. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C for up to 6 month. Avoid repeated freeze-thaw cycles.

Note: For serum/plasma: Fasting specimens or pre-feed specimens for children (2- 3 hours after last meal) are advised.

Haemolytic and especially lipemic samples should not be used for the assay.

REAGENT PREPARATION

- **1X Wash buffer**: Dilute 50X Wash buffer into distilled water to yield 1X Wash buffer. (E.g.: 20 ml of 50X Wash buffer + 980 ml of distilled water). The prepared wash buffer should be stored in 2 – 8 °C and used within 1 month after dilution.
- **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 coated 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.

ASSAY PROCEDURE

- Precipitation

1. Add **20 µl** of **standards, controls and samples** into the tubes.
2. Add **200 µl** of **PBS** into each tube.
3. Add **25 µl** of **Precipitating Reagent** into each tube.
4. **Mix** the reaction tubes (vortex) and centrifuge for **15 minutes at 3000 x g**.
5. Take **25 µl** of the clear supernatant for the **derivatization**.

- Derivatization

1. Add **25 µl** of **precipitated standards, controls and samples** in duplicate into the Reaction Plate.
2. Add **50 µl** of **Equalizing Reagent** into each well.
3. Add **10 µl** of the **D-Reagent** into each well.
4. **Cover the wells** with Plate sealer and incubate for **2 hours at RT** on a **shaker (600rpm)**.
5. Add **100 µl** of the **Q-Buffer** into all wells. **Shake 600rpm for 10 min. at RT.**
6. Use **25 µl** for the ELISA.

- Tryptophan ELISA

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

1. Add **25 µl** of the **derivatized standards, controls and samples** in duplicates into the **Tryptophan-coated microtiter strips**.
2. Add **50 µl** of the **Tryptophan antiserum** into each well.
3. **Cover the wells** with Plate sealer and incubate for **15-20 hours at 2-8°**C.
4. Aspirate each well and wash, repeating the process 2 times for a total **3**

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 the **Enzyme-Conjugate Antibody** into each well. Incubate for **30 min at RT** on microplate shaker **at ~600 rpm.**
6. Aspirate each well and **wash as step 4**.
7. Add **100 µl** of **TMB Reagent** to each well. Incubate for **20-30 minutes at room temperature in dark** on microplate shaker **at ~600 rpm**.
8. Add **100 µl** of **Stop Solution** to each well. Shake the microtiter plate to ensure a homogeneous distribution of the solution.
9. Read the OD with a microplate reader at **450 nm** within 10 minutes after adding STOP solution. (If available a reference wavelength between **620 nm and 650 nm** is recommended).

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using 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. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. 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.
5. Refer to the table below for molar conversion:

	Concentration of standards					
Standard	A	B	C	D	E	F
Tryptophan (µg/ml)	0	2.5	7.5	25	75	250
Tryptophan (µmol/L)	0	12.2	36.7	122	367	1224
Conversion	Tryptophan (µg/ml) x 4.89 = Tryptophan (µmol/L)					

6. Final calculation for urine samples:

   Calculate the 24 hours excretion for each urine sample:

   µg/24 h = µg/L x L/24 h

7. Expected reference values

It is strongly recommended that each laboratory should determine its own

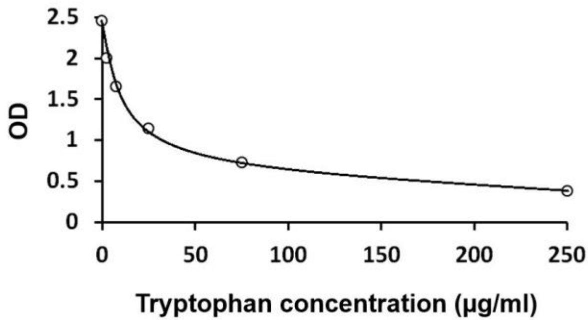
reference values.

Plasma / Serum: 9.3 – 17 µg/ml

Spontaneous urine: 1.5 – 40 µg/g creatinine

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Tryptophan ranged from 1.2-250 μg/ml. The mean MDD was 1.2 μg/ml

Specificity

<table>
<tr><td rowspan="7">Analytical Specificity</td><td>Substance</td><td>Cross Reactivity (%)</td></tr>
<tr><td>Tryptophan</td><td>100</td></tr>
<tr><td>5-Hdroxy-L-trytophan</td><td>< 0.01</td></tr>
<tr><td>Tryptamine</td><td>0.2</td></tr>
<tr><td>5-Methoxytrytophan</td><td>< 0.01</td></tr>
<tr><td>5-Hydroxytryptophan</td><td>< 0.01</td></tr>
<tr><td>N-acetyl-5-hydroxytryptamine</td><td>< 0.01</td></tr>
</table>

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 11 % and inter-assay precision was 11.7%.

Recovery

Urine: 104-110%

Serum: 86-100%

Linearity

Urine: 101- 129