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Instructions for use

Tryptophan ELISA

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

For research use only, not for use in diagnostic procedures.

REF

IB89127



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1. Introduction

1.1 **Intended use and principle of the test**

Enzyme Immunoassay for the determination of Tryptophan in urine, serum and plasma samples. For research use only, not for use in diagnostic procedures.

After extraction and derivatization Tryptophan is determined by ELISA.

The competitive ELISA uses the microtiter plate format. The antigen is bound to the solid phase of the microtiter plate. The acylated 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.

Determination of unknown samples is achieved by comparing their absorbance with a standard curve prepared with known standards.

1.2 **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.

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 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) This assay was validated for certain types of samples as indicated in *Intended Use* (please refer to Chapter 1). Any off-label use of this kit is in the responsibility of the user and the manufacturer cannot be held liable.
- (3) The principles of Good Laboratory Practice (GLP) have to be followed.
- (4) In order to reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves and protective glasses where necessary.
- (5) 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.
- (6) For dilution or reconstitution purposes, use deionized, distilled or ultra-pure water.
- (7) 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.
- (8) Duplicate determination of sample is highly recommended to be able to identify potential pipetting errors.
- (9) 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.
- (10) Incubation times do influence the results. All wells should be handled in the same order and time intervals.
- (11) To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
- (12) A standard curve must be established for each run.
- (13) The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report.
- (14) 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.
- (15) Avoid contact with Stop Solution containing 0.25 M H₂SO₄. It may cause skin irritation and burns. In case of contact with eyes or skin, rinse off immediately with water.
- (16) 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.
- (17) 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.

- (18) The expected reference values reported in this test instruction are only indicative. It is recommended that each laboratory establishes its own reference intervals.
- (19) The results obtained with this test kit are for research use only, not for use in diagnostic procedures.
- (20) Kit reagents must be regarded as hazardous waste and disposed of according to national 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 or which are haemolytic or lipemic might cause inaccurate results.

24-hour urine

Please note the sample preparation! If the percentage of the final concentration of acid is too high, the buffer capacity of the Diluent is insufficient. As a consequence tryptophan will not be extracted quantitatively.

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 test.

3. Storage and stability

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.

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

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 coloured
 Volume: 1 x 6 ml/vial, blue cap

Standards and Controls - Ready to use

| Cat. no. | Component | Colour/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-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-2446 **D-REAGENT** **D-Reagent** - Ready to use

Contents: Crosslinking agent in dimethylsulfoxide
 Volume: 1 x 4 ml/vial, white cap

Hazards identification: 

H318 Causes serious eye damage.
 H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.
 H332 Harmful if inhaled.
 H315 Causes skin irritation.
 H317 May cause an allergic skin reaction.

BA E-2721 **PREC-REAG** **Precipitating Reagent** - Ready to use

Contents: Acidic reagent for precipitation of plasma/serum proteins, red coloured

Volume: 1 x 4 ml/vial, white cap

Hazards
identification:



H302 Harmful if swallowed.

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

5. 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 at room temperature immediately after collection.

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.

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 venipuncture (Monovette™ or Vacuette™ for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Subjects receiving anticoagulant therapy may require increased clotting time.

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.

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. If the percentage of the final concentration of acid is too high, the buffer capacity of the Diluent is insufficient. As a consequence tryptophan will not be extracted quantitatively.

Storage: for longer periods (up to 6 month) at -20 °C.

Repeated freezing and thawing should be avoided. Avoid exposure to direct sunlight.

6. Test procedure

Allow all reagents and samples to reach room temperature and mix thoroughly by gentle inversion before use. Duplicate determinations are recommended.

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.



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

6.1 Preparation of 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 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.

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 h 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! |

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 h (overnight) at 2 – 8 °C . |
| 4. | Remove the foil. 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. |
| 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). |

7. Calculation of results

| | |
|------------------------|-------------------|
| Measuring range | Tryptophan |
| | 1.2 - 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 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:

$$\mu\text{g}/24\text{h} = \mu\text{g}/\text{l} \times \text{l}/24\text{h}$$

Conversion

$$\text{Tryptophan } (\mu\text{g}/\text{ml}) \times 4.89 = \text{Tryptophan } (\mu\text{mol}/\text{l})$$

Expected reference values

It is strongly recommended that each laboratory should determine its own reference values.

| Plasma / Serum | Spontaneous urine |
|----------------------------------|--|
| 9.3 – 17 $\mu\text{g}/\text{ml}$ | 1.5 – 40 $\mu\text{g}/\text{g}$ creatinine |

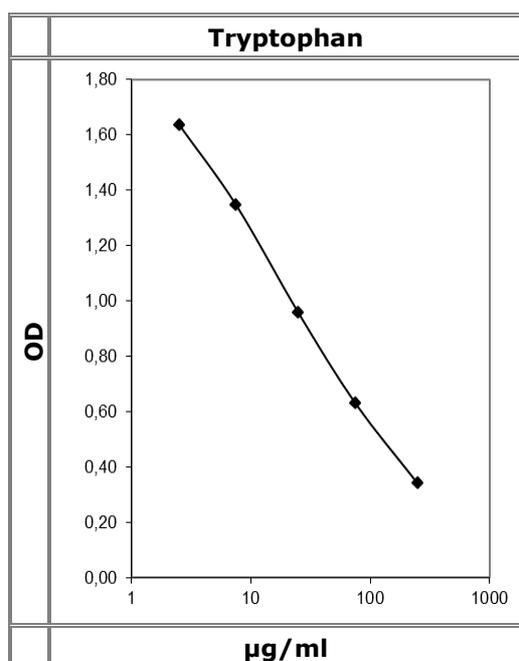
7.1 Quality control

It is recommended to use control samples according to national regulations. Use controls at both normal and elevated levels. The kit controls or other commercial controls should fall within established confidence limits. 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

| Analytical Sensitivity (Limit of Detection) | Tryptophan |
|--|-----------------------------|
| | 1.2 $\mu\text{g}/\text{ml}$ |

| 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 (n = 77) | 9.4 ± 1.0 | 11 | 1 (n = 16) | 9.2 ± 1.4 | 15 |
| 2 (n = 78) | 27 ± 2.8 | 11 | 2 (n = 16) | 45 ± 4 | 8.4 |

| Linearity | | Range (µg/ml) | Serial dilution up to | Range (%) |
|------------------|-------|---------------|-----------------------|-----------|
| | Urine | 1.3 - 100 | 1:75 | 101 - 129 |

| Recovery | | Mean (%) | Range (%) |
|-----------------|-------|----------|-----------|
| | Urine | 106 | 104 - 110 |
| | Serum | 95 | 86 - 100 |

9. References/Literature

- (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)

For orders, please contact:

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 **For updated literature or any other information please contact your local supplier.**

Symbols:

| | | | | | |
|---|------------------------------|---|------------------|---|-----------------------------------|
|  | Storage temperature |  | Manufacturer |  | Contains sufficient for <n> tests |
|  | Expiry date | LOT | Batch code | IVD | For in-vitro diagnostic use only! |
|  | Consult instructions for use | CONT | Content | CE | CE labelled |
|  | Caution | REF | Catalogue number | RUO | For research use only! |