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Instructions for use

Tryptophan ELISA

REF BA E-2700R

2°C – 8°C

Σ 96

RUO

For research use only – Not for use in diagnostic procedures

Table of contents

1. Introduction

1.1 Intended use and principle of the test

Enzyme immunoassay for the quantitative determination of L-tryptophan in urine, serum and EDTA-plasma samples for the determination of tryptophan homeostasis.
Tryptophan is present in the blood in protein-bound form. To isolate tryptophan, protein precipitation is first performed followed by a derivatization process. The subsequent competitive ELISA uses the microtiter plate format. The antigen is bound to the solid phase of the microtiter plate. The analyte concentrations of the standards, controls and samples compete with the solid phase bound analyte concentrations for a fixed number of antibody binding sites. After 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 resulting in a colour reaction. The reaction is monitored at a wavelength of 450 nm.
Quantification of unknown samples is achieved by comparing their absorbance with a reference curve prepared with known standard concentrations. Manual processing of the ELISA is recommended. The use of automatic laboratory equipment is the responsibility of the user.
This product is not intended to clinical diagnoses.

1.2 Background

The amino acid L-tryptophan is essential for humans [1-4] and is absorbed through the diet [1, 2, 5, 6]. Tryptophan serves as a precursor in the synthesis of the neurotransmitters serotonin [2, 4-6] and tryptamine [2, 6] and the epiphyseal hormone melatonin [4, 7], among others. The enzyme indoleamine-2,3-dioxygenase (IDO) converts tryptophan to kynurenine [2, 5, 6]. Increased IDO activity is a sign of immunological dysregulation in humans, which is often described with infections [8] or even neurodegenerative diseases [8]. Furthermore, tryptophan and its metabolites regulate neurobehavioral patterns and may affect well-being (depressive symptoms) [9, 10].

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 must 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) must 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. For dilution or reconstitution purposes, use deionized, distilled, or ultra-pure water. Avoid repeated freezing and thawing of reagents and specimens.
(5) The microplate contains snap-off strips. Unused wells must be stored at 2 – 8 °C in the sealed foil pouch with desiccant and used in the frame provided. Microtiter strips which are removed from the frame for usage should be marked accordingly to avoid any mix-up.
(6) Duplicate determination of sample is highly recommended.
(7) Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials, and devices are prepared for use at the appropriate time.
(8) Incubation times do influence the results. All wells should be handled in the same order and time intervals.
(9) To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
(10) A standard curve must be established for each run.
(11) The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report provided with the kit.
(12) 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.
(13) 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.
(14) 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. Rinse contaminated items before reuse.

(15) For information about 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 the website of the manufacturer or upon request.
(16) Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.
(17) In case of any severe damage to the test kit or components, the manufacturer has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components must not be used for a test run. They must be stored properly until the manufacturer decides what to do with them. If it is decided that they are no longer suitable for measurements, they must be disposed of in accordance with national regulations.

2.2 Limitations

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

2.2.1 Interfering substances and proper handling of specimens

Serum/Plasma

Samples containing precipitates or fibrin strands might cause inaccurate results. Hemolytic samples (up to 2 mg/ml hemoglobin), icteric samples (up to 0.5 mg/ml bilirubin) and lipemic samples (up to 16 mg/ml triglycerides) have no influence on the assay results.
If the concentrations cannot be estimated and there are doubts as to whether the above limit values for hemolytic, icteric or lipemic samples are complied with, the samples should not be used in the assay.

2.2.2 Drug and food interferences

There are no known substances (drugs) which ingestion interferes with the measurement of tryptophan level in the sample. Fasting specimens or pre-feed specimens for children (2 – 3 hours after last meal) are advised.

2.2.3 High-Dose-Hook effect

No hook effect was observed in this test.

3. Storage and stability

Store kit and reagents at 2 – 8 °C until expiration date. Do not use kit and components beyond the expiry date indicated on the kit labels. Once opened, the reagents are stable for 2 months when stored at 2 – 8 °C (except reagent BA E-2428, see chapter 6.1). Once the resealable pouch of the ELISA plate has been opened, care should be taken to close it tightly again including the desiccant.

4. Materials

4.1 Contents of the kit

BA D-0024	**REAC-PLATE**	**Reaction plate** – ready to use
Content:	1 x 96 well plate, empty in a resealable pouch	
BA D-0090	**FOILS**	**Adhesive Foil** – ready to use
Content:	Adhesive foils in a resealable pouch	
Number:	1 x 4 foils	
BA E-0030	**WASH-CONC** **50x**	**Wash Buffer Concentrate** – concentrated 50x
Content:	Buffer with a non-ionic detergent and physiological pH	
Volume:	1 x 20 ml/vial, purple cap	
BA E-0040	**CONJUGATE**	**Enzyme Conjugate** – ready to use
Content:	Goat anti-rabbit immunoglobulins conjugated with peroxidase	
Volume:	1 x 12 ml/vial, red cap	
Description:	Species is goat	
BA E-0055	**SUBSTRATE**	**Substrate** – ready to use
Content:	Chromogenic substrate containing 3,3',5,5'-tetramethylbenzidine, substrate buffer and hydrogen peroxide	
Volume:	1 x 12 ml/vial, black cap	
BA E-0080	**STOP-SOLN**	**Stop Solution** – ready to use
Content:	0.25 M sulfuric acid	
Volume:	1 x 12 ml/vial, grey cap	

BA E-2413	**ASSAY-BUFF**	**Assay Buffer** – ready to use
Content:	Buffer with alkaline pH	
Volume:	1 x 20 ml/vial, yellow cap	
Hazard pictograms:	 GHS08 GHS07	
Signal word:	Danger	
Hazardous ingredients:	Boric acid	
Hazard statements:	H360FD May damage fertility. Suspected of damaging the unborn child.	
Precautionary statements:	P201 Obtain special instructions before use. P280 Wear protective gloves, protective clothing, eye protection, face protection. P308+P313 IF exposed or concerned: Get medical advice/attention. P501 Dispose of contents/container to an authorised waste collection point.	
Additional statements:	Restricted to professional users.	
BA E-2428	**EQUA-REAG**	**Equalizing Reagent** – lyophilized
Content:	Lyophilized protein	
Volume:	1 vial, brown cap	
Description:	Species is bovine	
BA E-2446	**D-REAGENT**	**D-Reagent** – ready to use
Content:	Crosslinking agent in dimethylsulfoxide	
Volume:	1 x 3 ml/vial, white cap	
Hazard pictograms:	 GHS07	
Signal word:	Warning	
Hazardous ingredients:	Glutaraldehyde	
Hazard statements:	H317 May cause an allergic skin reaction.	
Precautionary statements:	P261 Avoid breathing mist/vapours/spray. P280 Wear protective gloves. P333+P313 If skin irritation or rash occurs: Get medical advice/attention. P501 Dispose of contents/container to an authorised waste collection point.	
BA E-2458	**Q-BUFFER**	**Q-Buffer** – ready to use
Content:	TRIS buffer	
Volume:	1 x 20 ml/vial, white cap	
BA E-2710	**AS** **TRYP**	**Tryptophan Antiserum** – ready to use
Content:	Rabbit anti-L-tryptophan antibody in buffer with proteins and non-mercury preservative, blue coloured	
Volume:	1 x 6 ml/vial, blue cap	
Description:	Species of antibody is rabbit, species of protein in buffer is bovine	

BA E-2721	PREC-REAG	**Precipitating Reagent** – ready to use
Content:	Acidic reagent for precipitation of plasma/serum proteins, red coloured	
Volume:	1 x 4 ml/vial, white cap	
Hazard pictograms:	 GHS05	
Signal word:	Danger	
Hazardous ingredients:	5-sulphosalicylic acid dihydrate	
Hazard statements:	H314 Causes severe skin burns and eye damage.	
Precautionary statements:	P280 Wear protective gloves, protective clothing, eye protection. P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P310 Immediately call a doctor, a POISON CENTER. P501 Dispose of contents/container to an authorised waste collection point.	
BA E-2731	TRYP	**Tryptophan Microtiter Strips** – ready to use
Content:	1 x 96 wells (12x8) antigen precoated microwell plate in a resealable blue pouch with desiccant	
BA E-2788	PBS	**PBS** – ready to use
Content:	Phosphate buffered saline	
Volume:	1 x 20 ml/vial, orange cap	

4.2 Calibration and Controls

Standards and Controls – ready to use

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

Conversion: tryptophan [µg/ml] x 4.89 = tryptophan [µmol/l]

Content: Acidic buffer with non-mercury stabilizer, spiked with a defined quantity of tryptophan.

4.3 Additional materials required but not provided in the kit

- Water (deionized, distilled, or ultra-pure)
- Absorbent material (paper towel)
- Polystyrene or polypropylene tubes and suitable rack

4.4 Additional equipment required but not provided in the kit

- Calibrated precision pipettes to dispense volumes between 10 – 200 µl; 12.5 ml
- 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)
- Vortex mixer
- Centrifuge

5. Sample collection, handling and storage

Plasma

Whole blood should be collected by venepuncture into centrifuge tubes containing EDTA as anticoagulant (Monovette or Vacuette for plasma) and centrifuge 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.
Hemolytic, 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 months) at < -15 °C.

Serum

Whole blood should be collected by venepuncture into centrifuge tubes (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. Samples of donors receiving anticoagulant therapy may require increased clotting time.
Fasting specimens or pre-feed specimens for children (2 – 3 hours after last meal) are advised.
Hemolytic, 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 months) at < -15 °C.

Urine

Spontaneous urine (second morning urine) stabilized with 10 µl 6 M HCl per 1 ml of urine sample can be used. The measurement results are related to the creatinine content of the sample.
Storage: up to 48 hours at 2 – 8 °C; up to 6 months at < -15 °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. Number the Reaction Plate and microwell plates (Microtiter Strips which are removed from the frame for usage should be marked accordingly to avoid any mix-up). Duplicate determinations are recommended.
The binding of the antisera and of the enzyme conjugate and the activity of the enzyme are temperature dependent. The higher the temperature, the higher the absorption values will be. Varying incubation times will have similar influences on the absorbance. The optimal temperature during the enzyme immunoassay is between 20 – 25 °C.
If the product is prepared in parts, unused wells in Reaction Plates should be covered to avoid contamination. After preparation, the used wells must be labelled to prevent double use.
During the overnight incubation at 2 – 8 °C with the antiserum, the temperature should be uniform all over the ELISA plate to avoid any drift and edge-effect.

⚠ *The use of a microtiter plate shaker with the following specifications is mandatory: shaking amplitude 3 mm; approx. 600 rpm. Shaking with differing settings might influence the results.*

6.1 Preparation of reagents and further notes

Wash Buffer

Dilute the 20 ml Wash Buffer Concentrate **WASH-CONC** **50x** with water to a final volume of 1000 ml.
Storage: 2 months at 2 – 8 °C

Equalizing Reagent

Reconstitute the **EQUA-REAG** with 12.5 ml of **ASSAY-BUFF**. Reconstituted Equalizing Reagent which is not used immediately has to be stored in aliquots for max. 2 months at < -15 °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 homogenous, crystal-free solution.
Storage: 2 months at 2 – 8 °C

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 Preparation of samples – 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** **PREC-REAG** to all tubes.
4.	**Mix** the tubes thoroughly (vortex) and centrifuge for **15 min** 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 respective wells of the **REAC-PLATE**.
2.	Add **50 µl** of the **Equalizing Reagent** into all wells.
3.	Add **10 µl** of the **D-REAGENT** into all wells.
4.	Cover plate with **FOILS** and incubate for **2 h** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm).
5.	Add **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).
⚠	**Use 25 µl for the ELISA!**

6.4 Tryptophan ELISA

1.	Pipette **25 µl** of the **derivatized standards, controls** and **samples** into the appropriate wells of the **TRYP**.
2.	Add **50 µl** of the **AS** **TRYP** into all wells and mix shortly.
3.	Cover plate with **FOILS** and incubate for **15 – 20 h** (overnight) at **2 – 8 °C**.
4.	Remove the **FOILS**. Discard or aspirate the contents of the wells. Wash the plate **3 times** by adding **300 µl** of **Wash Buffer**, **discarding** the content and **blotting dry each time** by tapping the inverted plate on absorbent material.
5.	Add **100 µl** of the **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 times** by adding **300 µl** of **Wash Buffer**, **discarding** the content and **blotting dry each time** by tapping the inverted plate on absorbent material.
8.	Add **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-SOLN** to each well and shake the microtiter plate shortly.
10.	**Read** the **absorbance** of the solution in the wells within 10 min, 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	0.73 – 250 µg/ml

The standard curve, which can be used to determine the concentration of the unknown samples, 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) using a concentration of 0.001 µg/ml for Standard A (this alignment is mandatory because of the logarithmic presentation of the data). Use non-linear regression for curve fitting (e.g. 4-parameter, marquardt).

⚠*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.*

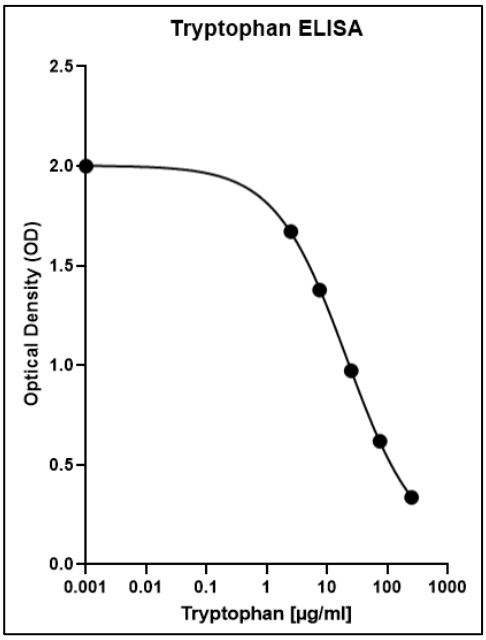
The concentrations of the samples and controls can be read directly from the standard curve.
Samples found with concentrations higher than the highest standard (Standard F) should be diluted accordingly with water (deionized, distilled, or ultra-pure) and must be re-assayed.

Tryptophan related to the creatinine content of the sample: **mg/g creatinine =** $\frac{\text{mg tryptophan}}{\text{l}} : \frac{\text{g creatinine}}{\text{l}}$

Conversion:
tryptophan [µg/ml] x 4.89 = tryptophan [µmol/l]

7.1 Typical standard curve

⚠ *Example: Do not use for calculation!*



8. Control samples

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

9. Assay characteristics

9.1 Performance data

Analytical Sensitivity	
Limit of Blank (LOB)	0.48 µg/ml
Limit of Detection (LOD)	0.65 µg/ml
Limit of Quantification (LOQ)	0.73 µg/ml

Analytical Specificity (Cross Reactivity)	
Substance	**Cross Reactivity [%]**
Tryptophan	100
5-Hydroxy-L-Tryptophan	< 0.01
5-Methoxy-L-Tryptophan	< 0.01
Tryptamine	< 0.01
5-Methoxytryptamine	< 0.01
5-Hydroxytryptamine	< 0.01

Precision					
Intra-Assay			**Inter-Assay**		
Sample	Mean ± SD [µg/ml]	CV [%]	Sample	Mean ± SD [µg/ml]	CV [%]
1	3.3 ± 0.9	26.7	1	2.8 ± 0.5	17.3
2	7.3 ± 1.1	14.7	2	7.7 ± 1.1	14.2
3	23.2 ± 2.2	9.3	3	23.4 ± 3.4	14.7
4	67.6 ± 4.4	6.4	4	66.4 ± 7.5	11.3

Lot-to-Lot			
	Sample	Mean ± SD [µg/ml]	CV [%]
Tryptophan in artificial matrix (n = 6)	1	6.5 ± 0.22	3.4
	2	31.5 ± 2.1	6.7
Tryptophan in plasma (n = 6)	1	10.1 ± 0.31	3.1
	2	48.5 ± 2.9	6.0

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

Linearity		
Serial dilution up to	Mean [%]	Range [%]
1:64	94	73 – 115

Method Comparison: ELISA vs. LC-MS/MS	LC-MS/MS = 1.06x – 2.9; R^2 = 0.99; n = 41

9.2 Metrological Traceability

The values assigned to the standards and controls of the Tryptophan ELISA are traceable to the weighing.

Standards and Controls	Uncertainty [%]
	1.2

Tryptophan ELISA	
Concentration [µg/ml]	Expanded Uncertainty [%] k = 2*
2.8	34.1
7.7	28.1
23.4	30.1
66.4	22.1

* This defines an interval about the measured result that will include the true value with a probability of 95%.

10. References/Literature

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

11. Changes

Version	Release Date	Chapter	Change
15.0-r	2023-04-25	1. 2.1 2.2.2 3. 4.1 5. 6. 7. 9.1 9.2 10. 11.	- Introduction updated - Procedural notes, guidelines and warnings updated - Drug and food interferences updated - Shelf life extended after opening - BA E-2446 white cap (old: brown cap), Volume: 3 ml - 24 h collection urine removed - Stability of Wash Buffer and Equalizing Reagent adjusted - Calculation of results specified - Lot-to-Lot added - Metrological traceability added - References/Literature updated - Chapter Changes added
16.0-r	2024-02-21	4.1	- Hazard labelling updated according to SDS

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Use-by date	LOT	Batch code		
	Consult instructions for use	CONT	Content		
	Caution	REF	Catalogue number		Distributor
	Date of manufacture			RUO	For research use only!