Instructions for use

Tryptophan ELISA

REF **BA E-2700**

IVD

CE

Tryptophan ELISA

1. Intended use and principle of the test

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

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 acylated standards, controls and samples and the solid phase bound analyte compete for a fixed number of antiserum binding sites. When the system is in equilibrium, free antigen and free antigen-antiserum 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 reference curve prepared with known standards.

2. Advice on handling the test

2.1 Reliability of the test results

In order to assure a reliable evaluation of the test results it must be conducted according to the instructions included and in accordance with current rules and guidelines (GLP, RILIBÄK, etc.). Special attention must be paid to control checks for precision and correctness during the test; the results of these control checks have to be within the norm range. In case of significant discrepancies between the pre-set assay characteristics of this test and the actual results please contact the manufacturer of the test kit for further instructions.
It is recommended that each laboratory establishes its own reference intervals. The values reported in this test instruction are only indicative.
The results obtained with this test kit should not be taken as the sole reason for any therapeutic consequence but have to be correlated to other diagnostic tests and clinical observations.

2.2 Complaints

In case of complaints please submit to the manufacturer a written report containing all data as to how the test was conducted, the results received and a copy of the original test printout. Please contact the manufacturer to obtain a reclamation form and return it completely filled in to the manufacturer.

2.3 Warranty

This test kit was produced according to the latest developments in technology and subjected to stringent internal and external quality control checks. Any alteration of the test kit or the test procedure as well as the usage of reagents from different charges may have a negative influence on the test results and are therefore not covered by warranty. The manufacturer is not liable for damages incurred in transit.

2.4 Disposal

Residual substances and/or all remaining chemicals, reagents and ready for use solutions, are special refuse. The disposal is subject to the laws and regulations of the federation and the countries. About the removal of special refuse the responsible authorities or refuse disposal enterprises inform. The disposal of the kit must be made according to the national official regulations. Legal basis for the disposal of special refuse is the cycle economic- and waste law.
The appropriate safety data sheets of the individual products are available on the homepage. The safety data sheets correspond to the standard: ISO 11014-1.

2.5 Interference

Do not mix reagents and solutions from different lots. Consider different transport and storage conditions. Inappropriate handling of test samples or deviations from the test regulation can the results affect. Use no kit components beyond the expiration date. Avoid microbiological contamination of the reagents and the washing water. Consider incubation periods and wash references.

2.6 Precautions

Observe the incubation periods and washing instructions. Never pipette by mouth and avoid contact of reagents and specimens with skin. No smoking, eating or drinking in areas where samples or kit test tubes are handled. When working with kit components or samples, always wear protective gloves and wash your hand thoroughly as soon as you have finished the work. Avoid spraying of any kind. Avoid any skin contact with reagents. Use protective clothing and disposable gloves. All steps have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes. Sodium azide could react with lead and copper tubes and may form highly explosive metal azide. When clearing up, rinse thoroughly with large volumes of water to prevent such formation.
All reagents of this testkit which contain human or animal serum or plasma have been tested and confirmed negative for HIV I/II, HbsAg and HCV by FDA approved procedures.
All reagents, however, should be treated as potential biohazards in use and for disposal.

3. Storage and stability

Store the reagents at 2 - 8 °C until expiration date. Do not use components beyond the expiration date shown on the kit labels.

4.1 Contents of the kit

BA D-0024	REAC-PLATE	**Reaction Plate**	1 x 96 wells	ready for use
BA D-0090	FOILS	**Adhesive Foil**	1 x 4	ready for use
BA E-0030	WASH-CONC 50x	**Wash Buffer Concentrate**	1 x 20 mL	concentrate, dilute content with dist. water to a final volume of 1000 mL
BA E-0040	CONJUGATE	**Enzyme Conjugate**	1 x 12 mL	ready for use, anti-rabbit IgG conjugated with peroxidase
BA E-0055	SUBSTRATE	**Substrate**	1 x 12 mL	ready for use, containing a solution of tetramethylbenzidine (TMB)
BA E-0080	STOP-SOLN	**Stop Solution**	1 x 12 mL	ready for use, containing 0.25 M H_2SO_4
BA E-2446	D-REAGENT	**D-Reagent**	1 x 4 mL	ready for use
BA E-2458	Q-BUFFER	**Q-Buffer**	1 x 20 mL	ready for use
BA E-2701	STANDARD A	**Standard A**	1 x 4 mL	ready for use
BA E-2702	STANDARD B	**Standard B**	1 x 4 mL	ready for use
BA E-2703	STANDARD C	**Standard C**	1 x 4 mL	ready for use
BA E-2704	STANDARD D	**Standard D**	1 x 4 mL	ready for use
BA E-2705	STANDARD E	**Standard E**	1 x 4 mL	ready for use
BA E-2706	STANDARD F	**Standard F**	1 x 4 mL	ready for use
BA E-2710	AS TRYP	**Tryptophan Antiserum**	1 x 6 mL	from rabbit, ready for use, blue coloured, blue screw cap
BA E-2413	ASSAY-BUFF	**Assay Buffer**	1 x 20 mL	ready for use
BA E-2721	PREC-REAG	**Precipitating Reagent**	1 x 4 mL	ready for use
BA E-2428	EQUA-REAG	**Equalizing Reagent**	1 x	lyophilized
BA E-2731	TRYP	**Tryptophan Microtiter Strips**	1 x 96 wells	12 strips, 8 wells each, break apart, precoated
BA E-2751	CONTROL 1	**Control 1**	1 x 4 mL	ready for use
BA E-2752	CONTROL 2	**Control 2**	1 x 4 mL	ready for use
BA E-2788	PBS	**PBS**	1 x 20 mL	ready for use

4.2 Additional materials and equipment required but not provided with the kit

- Calibrated variable precision micropipettes (e.g. 10-100 µL / 100-1000 µL)
- Polystyrene or polypropylene tubes and suitable rack
- Microtiter plate washing device
- ELISA reader capable of reading absorbance at 450 nm
- Shaker (shaking amplitude 3mm; approx. 600 rpm)
- Absorbent material (paper towel)
- Distilled water
- Vortex mixer

5. Sample collection and storage

Urine

Spontaneous or 24-hour urine, collected in a bottle containing 10-15 mL of 6 M HCl, should be used.
Storage: for a longer period (up to 6 months) at -20°C.
Repeated freezing and thawing should be avoided.

Plasma

EDTA-, Heparin- or Citrate- plasma. Do not use haemolytic or lipemic samples. Fasting specimens or pre-feed specimens for children are advised.
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
Haemolytic and especially lipemic samples should not be used with this assay. Fasting specimens or pre-feed specimens for children are advised.
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.

6. Test procedure

Allow all reagents and samples to reach room temperature. Duplicate determinations are recommended.

6.1 Preparation of reagents

Wash Buffer
Dilute the 20 mL Wash Buffer Concentrate with distilled water to a final volume of 1000 mL.
Storage: up to 6 months 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 aliquotes at -20°C and may be thawed only once.

6.2 Preparation of samples

The Tryptophan ELISA is a flexible test system for various biological sample types and volumes. It is not possible to give a general advice how to prepare the samples. However, the following basics should help the researcher to adapt the protocol to his specific needs:

- It is advisable to perform a Proof of Principle to determine the recovery of tryptophan in the samples. Prepare a stock solution of tryptophan. Add small amounts (to change the native sample matrix as less as possible) of the stock solutions to the sample matrix and check the recovery.
- The sample volume determines the sensitivity of this test. Determine the sample volume needed to determine tryptophan in your sample by testing different amounts of sample volumes.

If you need any support in establishing a protocol for your specific purposes, do not hesitate to contact the manufacturer directly!

6.3 Precipitation

1.	Pipette **20 µL** of **standards**, **20 µL** of **controls,** and **20 µL** of **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.

6.4 Derivatization

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

6.5 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 and discard. Discard or aspirate the contents of the wells and **wash** each well **3 times** thoroughly with **300 µL Wash Buffer**. Blot dry 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 contents of the wells and **wash** each well **3 times** thoroughly with **300 µL Wash Buffer**. Blot dry 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 sun light!***
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** and a reference wavelength between 620 nm and 650 nm.

7. Calculation of results

	Concentration of the 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	1 224
Conversion:	Tryptophan (µg/mL) x 4.89 = Tryptophan (µmol/L)					

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).

The concentrations of the samples and controls can be read directly from the standard curve.

7.1 Quality control

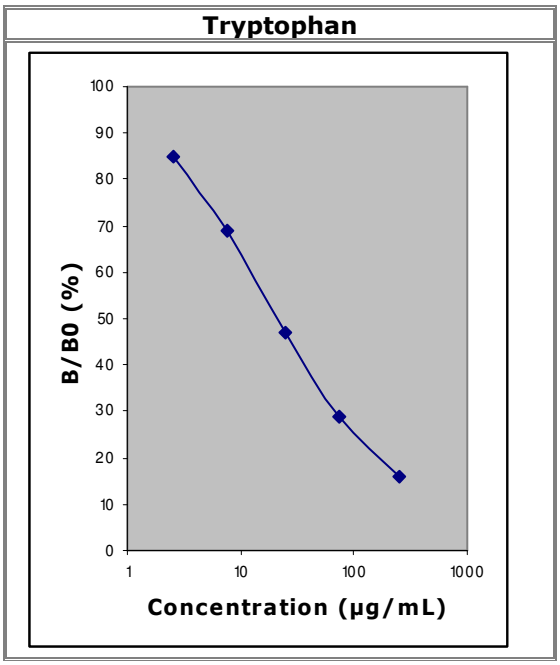
It is recommended to use control samples according to state and federal regulations. Use controls at both normal and pathological levels. The kit or other commercial controls should fall within established confidence limits. The confidence limits of the kit controls are indicated on the QC Report.

7.2 Calibration

The binding of the antisera and of the enzyme conjugate and the activity of the enzyme are temperature dependent, and the extinction 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*

7.3 Typical calibration curve (*example – do not use for calculation*)



8. Assay characteristics

Expected Reference Values		**Tryptophan**
	Urine	1.5 – 40 (µg/g creatinine; Adults > 18y)
	Plasma / Serum	9.3 – 17 (µg/mL; Adults > 16y)

Analytical Sensitivity (Limit of Detection)	**Tryptophan**
	1.2 µg/mL

Analytical Specificity (Cross Reactivity)	**Substance**	**Cross Reactivity (%)**
	Tryptophan	100
	5-Hydroxy-L-tryptophan	<0.01
	Tryptamine	0.2
	5-Methoxytryptophan	<0.01
	5-Hydroxytryptamine	<0.01
	N-acetyl-5-hydroxytryptamine	<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 ± 3	11	2 (n = 16)	45 ± 4	8.4

Linearity		Range	Serial dilution up to	Range (%)
	Urine	1.3 - 100	1:75	101 - 129
	Serum			

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

⚠ ***For current literature, information about clinical significance or any other information please contact your local supplier.***

Symbols:

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