



Instructions for Use

Serotonin High Sensitive ELISA


(high sensitivity and small sample volume)


Highly Sensitive Enzyme Immunoassay for the
Quantitative Determination of
Serotonin

RUO

For Research Use Only
Not for Use in Diagnostic Procedures

REF EA630/96

 12 x 8

 2 – 8 °C












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Symbols

 RUO	For Research Use Only		
 CONT	Content		Expiry Date
 LOT	Lot Number		Store at
	Manufactured by		Sufficient for n determinations
 REF	Catalogue Number		Consult Instructions for Use

The symbols of the components of the kit are described in chapter 4 Contents of the kit.

1 Introduction and Principle of the Test

The Serotonin High Sensitive ELISA provides materials for the quantitative measurement of derivated serotonin in low concentrated samples and for small sample volumes. The derivation is performed during the preparation of the samples. By using the acylation reagent the serotonin is quantitatively derivated into N-acylserotonin.

The Serotonin High Sensitive ELISA is a competitive ELISA kit in the microtiter plate format. Solid phase-bound serotonin and derivated serotonin in solution 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 serotonin is detected by anti-rabbit/peroxidase. The substrate TMB/peroxidase reaction is monitored at 450 nm. The amount of antibody bound to the solid phase serotonin is inversely proportional to the serotonin concentration of the sample.

2 Precautions




- For research use only. Not for use in diagnostic procedures.
- Before carrying out this test, the instructions for use, as included in the kit, should be completely read and the content understood.
- Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy but these materials should be handled as potentially infectious.
- Individual components of different lots and test kits should not be interchanged. The expiration dates and storage conditions indicated on the packaging and labels of individual components must be observed.
- When handling reagents, controls, and samples, follow established laboratory safety guidelines and Good Laboratory Practice.
- Wear protection, such as lab coat, appropriate gloves and eye protection.
- Some components of this kit contain hazardous reagents. These components are marked with the adequate hazard label. For further information, please see Chapter 4 Contents of the test kit and the relevant safety data sheets.
- Avoid any actions that could result in ingestion, inhalation or injection of the reagents. Never pipette by mouth.
- Avoid contact with individual reagents.
- Dispose of waste according to state and local environmental protection regulations.
- Broken glass can cause injury. Be careful with glass containers.



3 Storage and Stability

The kit is shipped at ambient temperature. Upon arrival, store the kit at 2 - 8 °C to keep it stable until its expiry date. Once opened the kit is stable until its expiry date. The shelf life of the ready-to-use reagents is indicated on the respective bottle label. For stability of prepared reagents refer to 6.1.

All reagents must equilibrate to room temperature before use and be refrigerated immediately after use.

4 Contents of the Kit

MT-Strips	STRIPS		12 strips
8 wells each, break apart, precoated with Serotonin			
Standard	CAL		1 vial
4 ml, concentrated, concentration: 500 ng/ml Dilute concentrate to working concentrations (see 6.1.2.)			
Control 1 & 2	CON 1 & CON 2		2 vials
Each 4 ml, concentrated, Dilute 1:500 (see 6.1.3.), Range: see QC certificate			
Acylation-Reagent	ACYL-REAG		4 vials
Lyoph., dissolve content in 2.5 ml SOLVENT (see 6.1.5.)			
Acylation Buffer	ACYL-BUFF		1 vial
Lyoph., dissolve content with 4 ml distilled water, add 200 µl of Acylation Buffer Concentrate ACYL-BUFF-CONC (see 6.1.4.)			
Acylation Buffer Concentrate	ACYL-BUFF-CONC		1 vial
1 ml, concentrated, colour coded yellow			Warning
Deactivator	DEAC		1 vial
3 ml, ready for use, colour coded blue			Warning
Enzyme Conjugate	CONJ		1 vial
12 ml, ready for use anti-rabbit-IgG-peroxidase			Warning
Wash Buffer	WASH		1 vial
20 ml, concentrated Dilute content with distilled water to 500 ml total volume (see 6.1.6.)			

Substrate 12 ml TMB solution, ready for use	SUB	1 vial
Stop Solution 12 ml, ready for use Contains 0.3 M sulphuric acid	STOP	1 vial
Solvent 12 ml, ready for use, Solvent to dissolve the Acylation Reagent, contains Acetone	SOLVENT	1 vial
		Warning
		Danger
Ascorbic Acid 2 ml, ready for use contains 10% ascorbic acid	ASC-ACID 10%	1 vial
Standard Buffer 50 ml, contains 10 mM PBS (0,9% NaCl), stabilized Before use enrich to 0.1% ascorbic acid (see 6.1.1.)	STD-BUFF	1 vial
Reaction plate For acylation, ready for use	ACYL-PLATE	1 piece
Adhesive foil Ready for use	FOIL	2 pieces

Additional materials and equipment required but not provided:

- Pipettes (10, 20, 25, 50, 100 and 200 µl)
- Orbital shaker
- Multichannel pipette or Microplate washing device
- Microplate photometer (450 nm and 570 – 650 nm)
- Distilled water
- Paper towels, pipette tips, timer

5 Sample Collection

The test is intended for small sample volumes, for low concentrated samples (e.g. tissue homogenates, dialysates) and in general for diluted samples.

To protect serotonin against oxidative degradation the samples must contain 0.1% ascorbic acid.

The samples can be stored up to 6 hours at 2 – 8 °C. For a longer storage the samples must be frozen at -20 °C. Repeated freezing and thawing should be avoided.

Different dilution buffers are suitable, but have to be tested beforehand. Evaluation was done with Ringer buffer and PBS (0.9% NaCl). Alternatively, Standard Buffer (see 6.1.1) included in the kit can be used. All applied buffers must contain 0.1% ascorbic acid.

For small sample volumes (< 20 µl) a volume correction is necessary. Add your dilution buffer (alternatively prepared Standard Buffer) to correct for volume.

For example:

Sample volume	Volume dilution buffer
1 µl	19 µl
2 µl	18 µl
5 µl	15 µl
10 µl	10 µl
15 µl	5 µl
20 µl	/

6 Preparation of Reagents and Samples

6.1 Preparation of Reagents

6.1.1 Standard Buffer

The Standard Buffer [STD-BUFF] has to be enriched to 0.1 % ascorbic acid prior use: e.g. 50 ml [STD-BUFF] + 0.5 ml [ASC-ACID 10%].

The prepared Standard Buffer should be stored at -20 °C and is stable until the expiration date printed on the vial label.

6.1.2 Standard

The concentration of [CAL] is 500 ng/ml (= 10.000 pg/sample) serotonin.

Dilute [CAL] to obtain working concentrations as follows:

Std 6	100 pg/sample	990 µl Dilution buffer	+	10 µl CAL
Std 5	20 pg/sample	800 µl Dilution buffer	+	200 µl Std 6
Std 4	6.7 pg/sample	933 µl Dilution buffer	+	67 µl Std 6
Std 3	2 pg/sample	980 µl Dilution buffer	+	20 µl Std 6
Std 2	0.67 pg/sample	993 µl Dilution buffer	+	6.7 µl Std 6
Std 1	0 pg/sample	1000 µl Dilution buffer		

For dilution of standards, use the same dilution buffer as present in the samples or as used for diluting the samples, respectively.

Alternatively, the prepared Standard Buffer (see 6.1.1) can be used for dilution of standards and samples.

All applied buffers must contain 0.1% ascorbic acid.

Dilution should be done in polypropylene (PP) tubes or polypropylene (PP) microtubes.

The diluted standards must be prepared shortly before use. After use, discard the standards.

6.1.3 Control 1 & 2

The controls **CON 1** & **CON 2** must be diluted 1:500 prior to use:

dil. Control 1 (1:500):	5,000 µl dilution buffer	+	10 µl CON 1
dil. Control 2 (1:500):	5,000 µl dilution buffer	+	10 µl CON 2

Use the same dilution buffer for controls as used for samples and standards.

The diluted controls must be prepared shortly before use.

6.1.4 Acylation Buffer

Dissolve the content of **ACYL-BUFF** with 4 ml distilled water. Add 200 µl of Acylation Buffer Concentrate **ACYL-BUFF-CONC**.

Mix shortly and leave on a roll mixer for 30 minutes. Handle carefully in order to minimize foam formation. The reconstituted Acylation Buffer should be stored frozen at -20 °C and is stable until the expiration date printed on the vial label.

6.1.5 Acylation Reagent

Dissolve the content of one bottle **ACYL-REAG** with 2.5 ml **SOLVENT** and shake for 5 minutes on an orbital shaker. The Acylation Reagent must be prepared immediately before use. After use, discard the reagent. The kit contains 4 vials allowing a maximum of 4 assay runs.

Please note that the solvent reacts with many plastic materials including plastic trays; solvent does not react with normal pipette tips and with glass devices.

Solvent is volatile and the dissolved Acylation Reagent evaporates quickly. Therefore, please do not use a tray with a large surface together with a multichannel pipette for pipetting the Acylation Reagent. Rather, use an Eppendorf multipipette (or similar device), fill the syringe directly from the vial with dissolved Acylation Reagent and add well by well.

6.1.6 Wash Buffer

Dilute the content (20 ml, 25x) of **WASH** with distilled water to a total volume of 500 ml, mix briefly.

For further use, the diluted wash buffer must be stored at 2 - 8 °C for a maximum period of 4 weeks.

If the kit is used in several runs, only the amount of wash buffer required for that run should be prepared.

All other reagents are ready for use.

6.2 Preparation of Samples (Acylation)

Allow reagents and samples to equilibrate to room temperature.

Determinations in duplicates are recommended.

The wells of the **ACYL-PLATE** can be used only once. Therefore, please mark the respective wells before use to avoid repeated use.

1. Pipette **25 µl prepared Acylation Buffer** (see 6.1.4) into the respective wells of **ACYL-PLATE**.
2. Add **20 µl each of diluted Standard 1 - 6, diluted Control 1 & 2 and Sample** to the respective wells.
3. Mix the reaction plate for 10 seconds on an orbital shaker.
4. Pipette **10 µl prepared Acylation Reagent** (see 6.1.5) into each well (colour changes to red) and continue with the next step, **immediately**.

Please note that solvent reacts with many plastic materials including plastic trays; solvent does not react with regular pipette tips and with glass devices.

Solvent is volatile and the dissolved Acylation Reagent evaporates quickly. Therefore, please do not use a tray with a large surface together with a multichannel pipette for pipetting Acylation Reagent. Rather, use an Eppendorf multipipette (or similar device), fill the syringe directly from the vial with dissolved Acylation Reagent and well by well.

5. Incubate for 60 minutes at room temperature on an orbital shaker at medium shaking frequency. Avoid direct sunlight.

Do not cover the wells or the plate; leave the plate open on the shaker.

6. Pipette **25 µl DEAC** into each well.
7. Cover the plate with **FOIL**.
8. Incubate for 3 hours at room temperature on an orbital shaker at medium shaking frequency. Avoid direct sunlight.

7 Test Procedure ELISA

1. Pipette **50 µl acylated Standards, Controls and Samples** into the respective wells of the **STRIPS**.
2. Cover the plate with **FOIL**, shake for 10 seconds on an orbital shaker at medium shaking frequency and incubate for 15 – 20 hours (overnight) at 2 - 8 °C.
3. Discard or aspirate the contents of the wells and wash thoroughly with **250 µl prepared Wash Buffer** (see 6.1.6) per well. **Discard or aspirate the contents of the wells and remove residual liquid by tapping the inverted plate on a clean absorbent paper.** Repeat the washing procedure **3** times.
4. Pipette **100 µl CONJ** into each well.
5. Incubate for 60 minutes at room temperature on an orbital shaker at medium shaking frequency.
6. Wash: Repeat step 3.
7. Pipette **100 µl SUB** into each well.
8. Incubate for 25 ± 5 minutes at room temperature (20 – 25 °C) on an orbital shaker at medium shaking frequency.
9. Pipette **100 µl STOP** into each well. Shake for 10 seconds on an orbital shaker.
10. Read the optical density at 450 nm (reference wavelength between 570 and 650 nm) in a microplate photometer within 15 minutes.

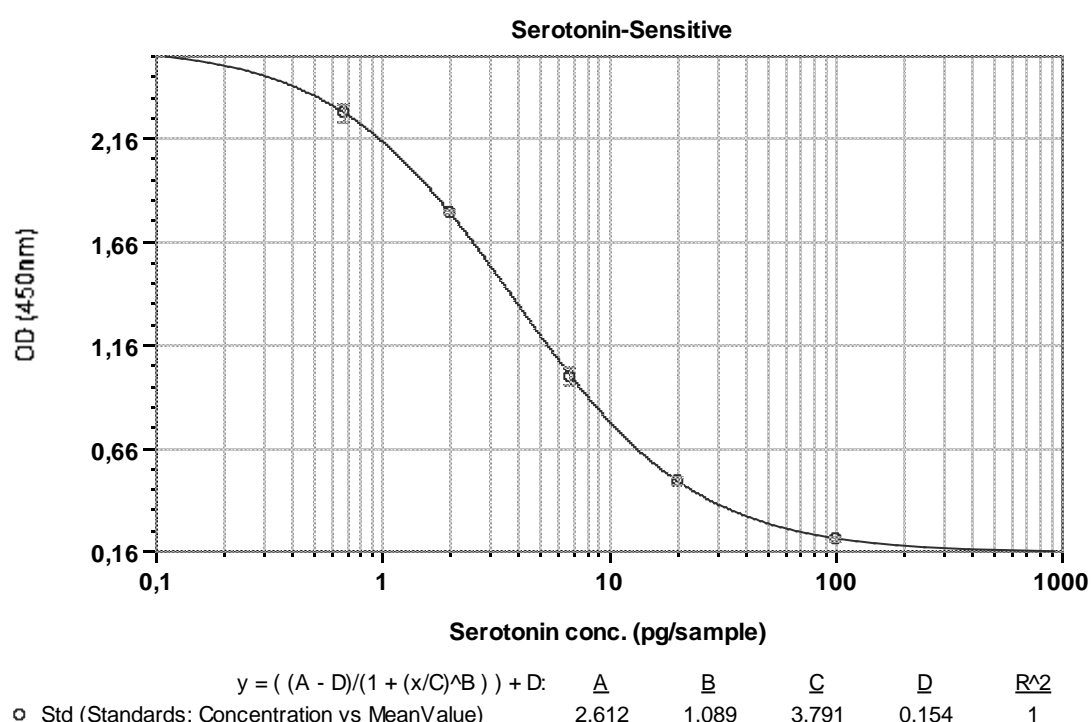
8 Calculation of the Results

The concentration of the standards (x-axis, logarithmic) are plotted against their corresponding optical density (y-axis, linear).

A good fit is provided with 4 Parameter Logistic (alternatively Log-Logit or Cubic Spline).

The concentration of the controls and samples (pg/sample) can be read directly from this standard curve by using their average optical density.

Example of a standard curve (do not use for calculation):



With a sample volume of 20 µl, 20 pg/sample correspond to 1 ng/ml (20 pg/20 µl sample). With a sample volume < 20 µl (see 5), that would be e.g. 20 pg/4 µl sample = 5 ng/ml. The standards are therefore: 5 ng/ml (100 pg/sample); 1 ng/ml (20 pg/sample); 0.335 ng/ml (6.7 pg/sample); 0.1 ng/ml (2 pg/sample); 0.0335 ng/ml (0.67 pg/sample).

Quality Control: Test results are valid only if the kit controls are within the ranges specified on the Quality Control Certificate. Otherwise, the test should be repeated.

9 Assay Characteristics

9.1 Analytical Sensitivity

The lower limit of detection was determined by taking the 2-fold standard deviation of the absorbance of the Zero Reference and reading the corresponding value from the standard curve.

Sensitivity : 0.39 pg/sample

9.2 Analytical Specificity (Cross Reactivity)

Structural related components were tested for possible interference with the antisera against serotonin used in the ELISA method.

Substance	ED-50-Value (ng/ml)	Cross Reactivity (%)
Serotonin	4.3	100
Tryptamine	1,996	0.22
5-Methoxytryptamine	17,083	0.025
5-Hydroxytryptophan	207,551	0.0021
Melatonin	677,434	< 0.001
5-HIAA	> 2,000,000	< 0.001
L-Tryptophan	> 20,000,000	< 0.0001

9.3 Reproducibility

The reproducibility of the ELISA method was investigated by determination of the intra-assay-coefficients of variation (cv) by repeated measurements of two samples with different serotonin concentrations.

Concentrations in **pg/sample**

Intra-Assay

Sample	n	Mean Value	sd	cv (%)
1	40	4.7	0.41	8.7
2	40	11.9	0.79	6.6

9.4 Calibration

The calibration is carried out by weighing the pure substance.

9.5 Limitations of the method

Results are for research purposes only.

Samples measured above the highest standard must be diluted and re-determined. Values of diluted samples must be multiplied by the appropriate dilution factor.

9.6 Interferences

No known interfering substances.

10 Changes to declare

Version 9: Changes are highlighted in gray.

Version 8: Changes are highlighted in gray.

Version 7: Changes in section 4 are highlighted in gray. Some wordings were revised to provide greater clarity.

Version 6: IFU has been re-formatted.

No changes have been made to components or execution of protocols.

Pipetting Scheme Sample Preparation

		Standards	Controls	Sample
ACYL-PLATE				
Prepared ACYL-BUFF	μl	25	25	25
Dil. CAL 1 – 6	μl	20		
Dil. CON 1 & CON 2	μl		20	
Sample	μl			20

Shake for 10 seconds

Freshly prepared ACYL-REAG	μl	10	10	10
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Incubate for 60 minutes at room temperature on an orbital shaker
 Do not cover wells or plate, leave the plate open on the shaker
 Avoid direct sunlight

DEAC	μl	25	25	25
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Cover plate with FOIL
 Incubate for 3 hours at room temperature on an orbital shaker.
 Avoid direct sunlight

Pipetting Scheme ELISA

		Acyl. Standard	Acyl. Control	Acyl. Sample
Transfer from ACYL-PLATE into STRIPS	µl	50	50	50

Cover plate with FOIL
 Shake for 10 seconds
 Incubate for 15 - 20 hours (overnight) at 2 – 8 °C
 4 x washing (with 250µl each)

CONJ	µl	100	100	100
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Incubate 60 minutes at room temperature on an orbital shaker
 4 x washing (with 250µl each)

SUB	µl	100	100	100
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Incubate 25 ± 5 minutes at room temperature on an orbital shaker

STOP	µl	100	100	100
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Shake for 10 seconds
 Read absorbance at 450 nm (ref. 570 – 650 nm)