DIAGNOSTIKA GMBH

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
Σ	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

Hazard Pictograms

 Danger

 Warning

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 competitive ELISA kit uses the microtiter plate format. Derivated serotonin 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.
- Disposable gloves and safety glasses should be used.
- 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.
- Some components of this kit are containing hazardous reagents. These components are marked with the adequate hazard label.

3 Storage and Stability

On arrival, store the kit at 2-8 °C. Once opened the kit is stable until its expiry date. For stability of prepared reagents refer to Preparation of Reagents.

Do not use components beyond the expiration date shown on the kit labels.

Do not mix various lots of any kit component within an individual assay.

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
Mix carefully in order to minimize formation of foam (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

Enzyme Conjugate **CONJ** 1 vial
12 ml, ready for use
anti-rabbit-IgG-peroxidase

Wash Buffer **WASH** 1 vial
20 ml, concentrated
Dilute content with distilled water to 500 ml total volume (see 6.1.6.)

Substrate **SUB** 1 vial
12 ml TMB solution, ready for use

Stop Solution **STOP** 1 vial
12 ml, ready for use
Contains 0.3 M sulphuric acid

Solvent **SOLVENT** 2 vials
6 ml, ready for use,
Solvent to dissolve the Acylation Reagent, contains Acetone


Warning


Danger

Ascorbic Acid **ASC-ACID 10%** 1 vial
2 ml, ready for use
contains 10% ascorbic acid

Standard Buffer **STD-BUFF** 1 vial
50 ml, contains 10 mM PBS (0,9% NaCl), stabilized
Before use enrich to 0.1% ascorbic acid (see 6.1.1.)

Reaction plate **ACYL-PLATE** 1 piece
For acylation, ready for use

Adhesive foil **FOIL** 2 pieces
Ready for use

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

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, STD-BUFF 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 for a minimum of 1 year.

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		

Use the same dilution buffer for dilution of standards as present in the samples or used for diluting the samples.

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

All applied buffers (also Standard Buffer) must contain 0.1% ascorbic acid.

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

The diluted standards must be prepared immediately 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, samples and standards.

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 big surface together with a multichannel pipette for pipetting the Acylation Reagent. Rather, use an Eppendorf multipette (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 of WASH with distilled water to a total volume of 500 ml.

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

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** into the respective wells of ACYL-PLATE.
2. Pipette each **20 µl diluted Standard 1 - 6, diluted Control 1 & 2 and Sample** into the respective wells.
3. Mix the reaction plate for 10 seconds.
4. Pipette **10 µl prepared Acylation Reagent** into each well (colour changes to red) and continue with step 5., **immediately.**

   Please note that 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 big surface together with a multichannel pipette for pipetting Acylation Reagent. Rather, use an Eppendorf multipette (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. 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. Avoid direct sunlight.

7 Test Procedure ELISA

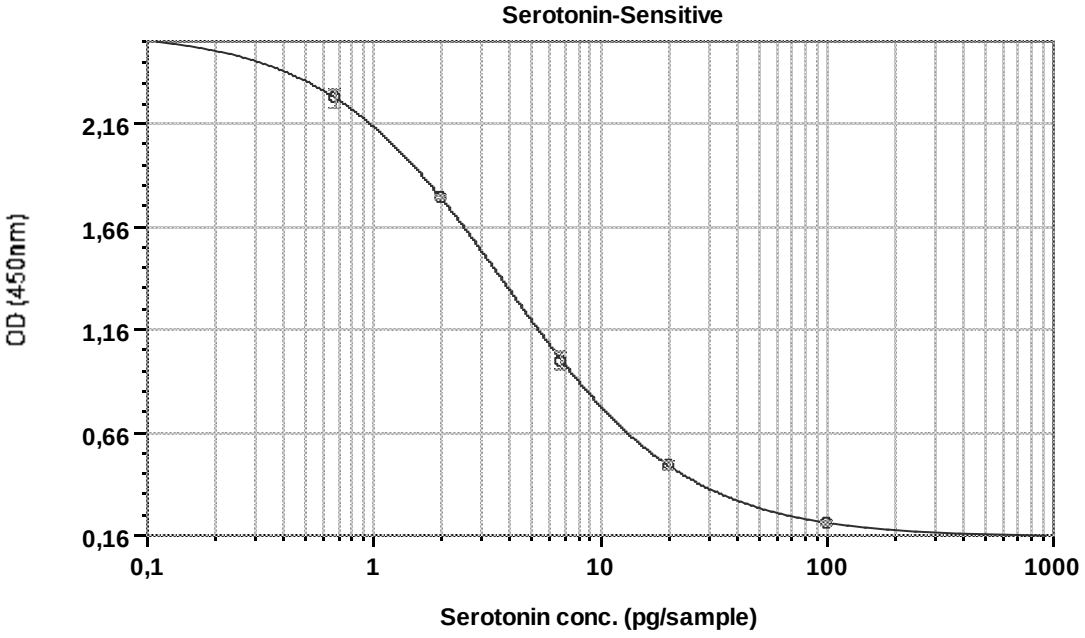
1. Pipette **50 µl acylated Standards 1 to 6**, **Controls** and **Samples** into the respective wells of the STRIPS.
2. Cover the plate with FOIL and incubate for 15 – 20 hours (overnight) at 2 - 8 °C.
3. Discard or aspirate the contents of the wells and wash thoroughly with each **300 µl prepared Wash Buffer**. Repeat the washing procedure 3 to 4 times. Remove residual liquid by tapping the inverted plate on clean absorbent paper.
4. Pipette **100 µl** CONJ into each well.
5. Incubate for 60 minutes at room temperature on an orbital shaker.
6. Wash: Repeat step 3.
7. Pipette **100 µl** SUB into each well.
8. Incubate for 20 to 30 minutes at room temperature (20 – 25 °C) on an orbital shaker.
9. Pipette **100 µl** STOP into each well.
10. Read the optical density at 450 nm (reference wavelength between 570 and 650 nm) in a microplate photometer within 10 minutes.

8 Calculation of the Results

The concentration of the standards (x-axis, logarithmic) are plotted against their corresponding optical density (y-axis, linear). Alternatively, the optical density of each standard and sample can be related to the optical density of the zero standard, expressed as the ratio OD/OD_{max}, and then plotted on the y-axis.

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

Typical standard curve:



y = ((A - D)/(1 + (x/C)^B)) + D:	A	B	C	D	R^2
Std (Standards: Concentration vs MeanValue)	2,612	1,089	3,791	0,154	1

9 Assay Characteristics

9.1 Sensitivity

The lower limit of detection was determined by taking the 2fold 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 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 ng/ml

Intra-Assay

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

10 Changes to declare

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. Standard 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

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

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

Pipetting Scheme ELISA

		Standard	Control	Sample
STRIPS				
Acyl. Standard 1 – 6	µl	50		
Acyl. CON 1 & CON 2	µl		50	
Acyl. Sample	µl			50

Cover plate with FOIL
Incubate for 15 – 20 hours (overnight) at 2-8°C
3 - 4 x washing

CONJ	µl	100	100	100

60 minutes incubation at room temperature on an orbital shaker
3 - 4 x washing

SUB	µl	100	100	100

20 - 30 minutes incubation at room temperature on an orbital shaker

STOP	µl	100	100	100

Reading of absorbance at 450 nm (ref. 570 – 650 nm)