DIAGNOSTIKA GMBH

Instructions for Use

Serotonin High Sensitive ELISA

(for High Sensitivity and for Small Sample Volume)

Highly Sensitive Enzyme Immunoassay
for the Quantitative Determination of Serotonin

REF EA 630/96

Σ 12 x 8

+2 +8 °C 2 – 8 °C

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Contents

Symbols

I V D	In-Vitro-Diagnostic Device	CE	EC Declaration of Conformity
CONT	Contents		Expiry Date
LOT	Lot Number	+2 +8 °C	Store
	Manufactured by	Σ	Sufficient for
REF	Catalogue Number		Consult Instructions

Hazard Pictograms

Danger

Warning

1. Introduction and Principle of the Test

Serotonin (5-Hydroxytryptamine), a biogenic amine, is a product of the tryptophan metabolism. It is a well evaluated neurotransmitter of the central nervous system and can be found in high concentrations in the chromaffine cells of the intestinal mucosa, in the platelets and the serotonergic neurones of the brain.

Central-serotonergic neurones influence physiological functions such as sleep and the hormonal and cardio-vascular regulation. Increased serum levels can be found with malignant carcinoid, endogenous depression and schizophrenia.

The assay kit 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 Serotonin ELISA kit uses the microtitre 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 in vitro use only.
- 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.

4. Contents of the Kit

4.1	**MT-Strips** 8 wells each, break apart precoated with serotonin	**STRIPS**	12 strips
4.2	**Standard** 4 ml, concentrated concentration: 500 ng/ml Dilute concentrate to working concentrations (see 6.1.2.)	**CAL**	1 vial
4.3	**Control 1 & 2** Each 4 ml, concentrated Dilute 1:500 (see 6.1.3.) Range: see q.c. certificate	**CON 1 & 2**	2 vials

4.4 **Acylation Buffer** **ACYL-BUFF** 1 vial
lyophilised, dissolve content with 4 ml distilled water,
add 200 µl of Acylation Buffer concentrate ACYL-BUFF-CONC
Mix carefully in order to minimize foam formation. (see 6.1.4.)

4.5 **Acylation Buffer Concentrate** **ACYL-BUFF-CONC** 1 vial
1 ml, concentrated; colour coded yellow


Warning

4.6 **Acylation Reagent** **ACYL-REAG** 2 x 2 vials
lyophilised, dissolve content
in 2.5 ml Solvent (see 6.1.5.)

4.7 **Deactivator** **DEAC** 1 vial
3 ml, ready for use; colour coded blue

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

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

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

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

4.12 **Solvent** **SOLVENT** 2 vials
6 ml solvent to dissolve the Acylation reagent
Contains Acetone, ready for use


Warning 
Danger

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

4.14 **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.).

4.15 **Reaction plate** **ACYL-PLATE** 1 piece
for Acylation, ready for use

4.16 **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)
- 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.
For the protection of 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, the Standard Buffer 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 dilution buffer (alternatively 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 **STD-BUFF**

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

The prepared Standard Buffer should be stored frozen at -20 °C and is stable for a minimum of 1 year.

6.1.2.Standard **CAL**

The concentration of the standard is 500 ng/ml (= 10.000 pg/sample) serotonin.
Dilute Standard for obtaining working concentrations as follows:
0 / 0,67 / 2 / 6,7 / 20 / 100 pg/sample.

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 (100 pg/sample)**
Std 4	**6.7 pg/sample**	933 µl Dilution buffer	67 µl **Std 6 (100 pg/sample)**
Std 3	**2 pg/sample**	980 µl Dilution buffer	20 µl **Std 6 (100 pg/sample)**
Std 2	**0.67 pg/sample**	993 µl Dilution buffer	6.7 µl **Std 6 (100 pg/sample)**
Std 1	**0 pg/sample**	1000 µl Dilution buffer	

Use the same dilution buffer for dilution of standards, sample and for the experiment.
Alternatively, the Standard Buffer can be used for dilution of standards and samples.
All applied buffers must contain 0.1% ascorbic acid (Standard Buffer, too).

Dilution should be done in polypropylene (PP) tubes or polypropylene (PP) microtubes.
The diluted standards have always to be prepared immediately before use. After use they have to be discarded.

6.1.3. Kontrolle 1 & 2 **CON 1 & 2**

The controls have to be diluted 1:500 prior to use:

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

Use the same dilution buffer for controls and standards.

6.1.4. Acylation Buffer **ACYL-BUFF**

Dissolve the content 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 **ACYL-REAG**

Dissolve the content of one bottle in 2.5 ml Solvent and shake for 5 minutes on an orbital shaker. The Acylation Reagent has always to be prepared immediately before use. After use the reagent has to be discarded. The kit contains 4 vials allowing for a maximum of 4 assay runs.

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 add well by well.

6.1.6. Wash Buffer **WASH**

Dilute the content 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 reach room temperature.
Determinations in duplicates are recommended.

The wells of the reaction plate for the acylation can be used only once. Therefore, please mark the respective wells before use.

1. Pipette each **25 µl Acylation Buffer** into the respective wells of the reaction 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 each **10 µl Acylation Reagent** into all wells (colour changed 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 each **25 µl Deactivator** into all wells.

7. Cover the plate with adhesive foil.
   Incubate for 3 hours at room temperature on an orbital shaker. Avoid direct sunlight.

   Take each 50 µl for the ELISA.

7. Test Procedure ELISA

Allow reagents and samples to reach room temperature.
Determinations in duplicates are recommended.

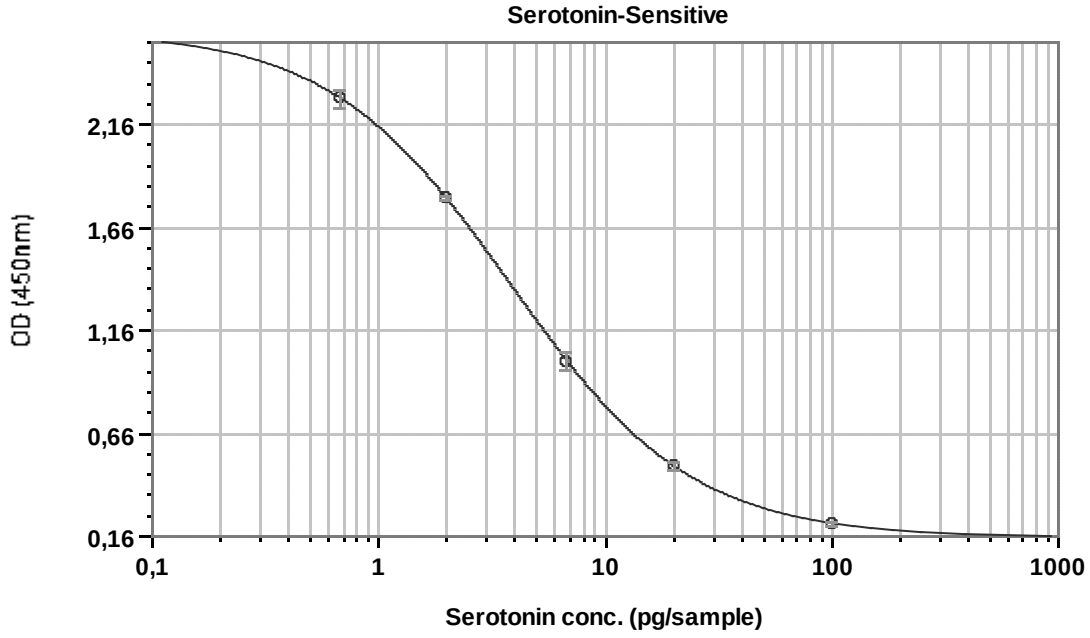
1. Pipette each **50 µl prepared Standards 1 to 6**, **controls** and **Samples** into the respective wells of the coated microtiter strips
2. Cover the plate with adhesive foil and incubate for 15 – 20 hours (overnight) at 2-8°C.
3. Discard or aspirate the contents of the wells and wash thoroughly with each **300 µl Wash Buffer**. Repeat the washing procedure 3 to 4 times. Remove residual liquid by tapping the inverted plate on clean absorbent paper.
4. Pipette each **100 µl Enzyme Conjugate** into all wells.
5. Incubate for 60 minutes at room temperature on an orbital shaker.
8. Washing: Repeat step 3.
9. Pipette each **100 µl Substrate** into all wells.
10. Incubate for 20 to 30 minutes at room temperature (20 °C - 25 °C) on an orbital shaker.
11. Pipette each **100 µl Stop Solution** into all wells.
12. 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

On a semilogarithmic graph paper 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. Literatur

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Pipetting Scheme Sample Preparation

		Standard	Control	Sample
Acyl. Buffer	µl	25	25	25
Dil. Standard 1- 6	µl	20		
Dil. Control 1 & 2	µl		20	
Sample	µl			20

Shake for 10 seconds

Freshly prepared Acyl. Reagent	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

Deactivator	µl	25	25	25

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

take each 50 µl for the ELISA

Pipetting Scheme ELISA

		Standard	Control	Sample
Standard 1- 6	µl	50		
Control 1 & 2	µl		50	
Sample	µl			50

Cover plate with adhesive foil
Incubate for 15 – 20 hours (overnight) at 2-8°C

3 - 4 x washing

Enzyme Conjugate	µl	100	100	100

60 minutes incubation at room temperature on an orbital shaker

3 - 4 x washing

Substrate	µl	100	100	100

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

Stop Solution	µl	100	100	100

Reading of absorbance at 450 nm