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 | 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** **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** 1 vial
12 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
- 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 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 (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 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 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.

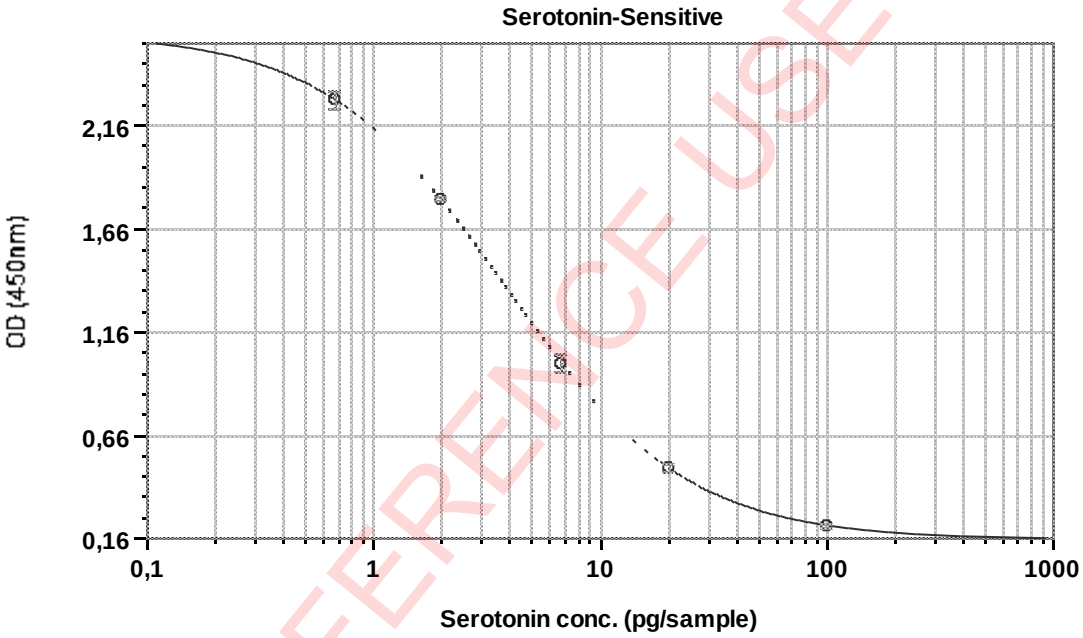
## 8 Calculation of the Results

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):




| | 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 |

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 |

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

| | | 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 |

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

| | | | | |
|---|---|---|---|---|
| SUB | µl | 100 | 100 | 100 |

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

| | | | | |
|---|---|---|---|---|
| STOP | µl | 100 | 100 | 100 |

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