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Instructions for use

SEROTONIN high sensitive ELISA

REF **BA E-5900R**

2°C – 8°C

Σ 96

RUO

For research use only – Not for use in diagnostic procedures

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1. Intended use and principle of the test

Ultra-sensitive enzyme immunoassay for the quantitative determination of Serotonin. Flexible test system for various biological sample types and volumes.
Serotonin is acylated and detected by the subsequent competitive ELISA kit, which uses the microtiter plate format. The antigen is bound to the solid phase of the microtiter plate. The acylated standards, controls and samples compete with the solid phase bound analytes for a fixed number of antibody binding sites. After the system is in equilibrium, free antigen and free antigen-antibody 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 resulting in a colour reaction. The reaction is monitored at a wavelength of 450 nm.
Quantification of unknown samples is achieved by comparing their absorbance with a reference curve prepared with known standard concentrations.

2. Procedural cautions, guidelines and warnings

(1) This kit is intended for professional use only. Users should have a thorough understanding of this protocol for the successful use of this kit. Only the test instruction provided with the kit is valid and must be used to run the assay. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
(2) The principles of Good Laboratory Practice (GLP) must be followed.
(3) In order to reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves and protective glasses where necessary.
(4) All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. For dilution or reconstitution purposes, use deionized, distilled, or ultra-pure water. Avoid repeated freezing and thawing of reagents and specimens.
(5) The microplate contains snap-off strips. Unused wells must be stored at 2 – 8 °C in the sealed foil pouch with desiccant and used in the frame provided. Microtiter strips which are removed from the frame for usage should be marked accordingly to avoid any mix-up.
(6) Duplicate determination of sample is highly recommended.
(7) Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials, and devices are prepared for use at the appropriate time.
(8) Incubation times do influence the results. All wells should be handled in the same order and time intervals.
(9) To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
(10) A standard curve must be established for each run.
(11) The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report provided with the kit.
(12) Do not mix kit components with different lot numbers within a test and do not use reagents beyond expiry date as shown on the kit labels.
(13) For information about hazardous substances included in the kit please refer to Safety Data Sheet (SDS). The Safety Data Sheet for this product is made available directly on the website of the manufacturer or upon request.
(14) Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.
(15) In case of any severe damage to the test kit or components, the manufacturer has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components must not be used for a test run. They must be stored properly until the manufacturer decides what to do with them. If it is decided that they are no longer suitable for measurements, they must be disposed of in accordance with national regulations.

3. Storage and stability

Store kit and reagents at 2 – 8 °C until expiration date. Do not use kit and components beyond the expiry date indicated on the kit labels. Once opened, the reagents are stable for 2 months when stored at 2 – 8 °C. Once the resealable pouch of the ELISA plate has been opened, care should be taken to close it tightly again including the desiccant.

4. Materials

4.1 Contents of the kit

BA D-0090	**FOILS**	**Adhesive Foil** – ready to use
Content:	Adhesive foils in a resealable pouch	
Number:	1 x 4 foils	
BA E-0030	**WASH-CONC** **50x**	**Wash Buffer Concentrate** – concentrated 50x
Content:	Buffer with a non-ionic detergent and physiological pH	
Volume:	1 x 20 ml/vial, purple cap	

BA E-0040	**CONJUGATE**	**Enzyme Conjugate** – ready to use
Content:	Goat anti-rabbit immunoglobulins conjugated with peroxidase	
Volume:	1 x 12 ml/vial, red cap	
Description:	Species is goat	
Hazard pictograms:	 GHS07	
Signal word:	Warning	
Hazardous ingredients:	2-methyl-2H-isothiazol-3-one	
Hazard statements:	H317 May cause an allergic skin reaction.	
Precautionary statements:	P280 Wear protective gloves. P302+P352 IF ON SKIN: Wash with plenty of water. P333+P313 If skin irritation or rash occurs: Get medical advice/attention. P501 Dispose of contents/container to an authorised waste collection point.	
BA E-0055	**SUBSTRATE**	**Substrate** – ready to use
Content:	Chromogenic substrate containing 3,3’,5,5’-tetramethylbenzidine, substrate buffer and hydrogen peroxide	
Volume:	1 x 12 ml/vial, black cap	
BA E-0080	**STOP-SOLN**	**Stop Solution** – ready to use
Content:	0.25 M sulfuric acid	
Volume:	1 x 12 ml/vial, grey cap	
BA E-0931	**IU SER 5-HIAA**	**Serotonin Microtiter Strips** – ready to use
Content:	1 x 96 wells (12x8) antigen precoated microwell plate in a resealable pouch with desiccant	
BA E-5910	**SER-AS**	**Serotonin Antiserum** – ready to use
Content:	Rabbit anti-serotonin antibody, blue coloured	
Volume:	1 x 3 ml/vial, blue cap	
Description:	Species is rabbit	
BA E-5911	**ACYL-BUFF**	**Acylation Buffer** – ready to use
Content:	TRIS buffer with non-mercury preservative	
Volume:	1 x 4 ml/vial, yellow cap	
BA E-5934	**ACYL-PLATE**	**Acylation Plate** – ready to use
Content:	1 x 96 well plate, pre-coated with acylation reagent	
BA E-5937	**STABILIZER**	**Stabilizer** – ready to use
Content:	Stabilizing agent, 10% solution	
Volume:	1 x 4 ml/vial, purple cap	
BA E-5941	**DIL-CONC 20x**	**Dilution Concentrate** – concentrated 20x
Content:	TRIS buffer with 1% stabilizing agent and a non-mercury preservative	
Volume:	1 x 50 ml/vial, white cap	

4.2 Calibration and Controls

Standards and **Controls** – concentrated*[1]

Cat. no.	Component	Colour/ Cap	Concentration after dilution ng/ml	nmol/l	pg/sample vol.	pmol/sample vol.	Volume/ Vial
BA R-8901	**STANDARD A**	white	0	0	0	0	4 ml
BA R-8902	**STANDARD B**	yellow	0.015	0.085	1.5	8.5	4 ml
BA R-8903	**STANDARD C**	orange	0.05	0.28	5	28.4	4 ml
BA R-8904	**STANDARD D**	blue	0.15	0.85	15	85	4 ml
BA R-8905	**STANDARD E**	grey	0.5	2.8	50	284	4 ml
BA R-8906	**STANDARD F**	black	2.5	14	250	1,418	4 ml
BA R-8951	**CONTROL 1**	green	Refer to QC-Report for expected value and acceptable range.				4 ml
BA R-8952	**CONTROL 2**	red					4 ml

Conversion: serotonin [ng/ml] x 5.67 = serotonin [nmol/l]
serotonin [pg/sample volume] x 5.67 = serotonin [pmol/sample volume]

Content: TRIS buffer with non-mercury preservative, spiked with defined quantity of serotonin

⚠ **1 Standards and controls have to be diluted 1+1000 prior to use (please see chapter 6.1 for further information). The shown concentrations apply to diluted standards and controls (1+1000) when 100 µl of undiluted sample is used. Please refer to chapter 7 for the use of a correction factor in case of using diluted samples and/or less sample volume.*

4.3 Additional materials required but not provided in the kit

- Water (deionized, distilled, or ultra-pure)
- Absorbent material (paper towel)

4.4 Additional equipment required but not provided in the kit

- Calibrated precision pipettes to dispense volumes between 1 – 100 µl; 10 ml
- Microtiter plate washing device (manual, semi-automated or automated)
- ELISA reader capable of reading absorbance at 450 nm and if possible 620 – 650 nm
- Microtiter plate shaker (shaking amplitude 3 mm; approx. 600 rpm)
- Vortex mixer

5. Sample collection and storage

In general, this assay is dedicated for any biological sample such as serum, urine, platelets, platelet-poor plasma, tissue homogenates, dialysates, and other samples.
Storage: up to 6 hours at 2 – 8 °C, for longer periods (up to 6 months) at -20 °C. Avoid exposure to direct sunlight.

Serum
Collect blood by venipuncture, allow to clot, and separate serum by centrifugation according to manufacturer's instructions at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.
Hemolytic and especially lipemic samples should not be used for the assay.

Urine
Spontaneous or 24-hour urine, collected in a bottle containing 10 – 15 ml of 6 M HCl, should be used.
Determine the total volume of urine excreted during a period of 24 h for calculation of the results.

Platelets
More than 98 percent of the circulating serotonin is located in the platelets and is released during blood clotting. Blood must be collected according to manufacturer's instructions by venipuncture in plastic tubes containing EDTA or citrate as anticoagulant.
To obtain platelet-rich plasma (PRP) the samples are centrifuged for 10 minutes at room temperature (200 x g). Transfer the supernatant to another tube and count the platelets.

The platelet pellet is obtained by adding 800 µl of physiological saline to 200 µl of PRP (containing between 350,000 – 500,000 platelets/µl) and centrifugation (4,500 x g, 10 minutes at 4 °C). The supernatant is then discarded.
200 µl of water (deionized, distilled, or ultra-pure) is added to the pellet and mixed thoroughly on a vortex mixer. This suspension can be stored frozen for several weeks at < -20 °C.

After thawing of the frozen samples, centrifuge at 10,000 x g for 2 minutes at room temperature.

⚠ ***To protect Serotonin against oxidative degradation the samples should contain 1% Stabilizer REF BA E-5937.***

6. Test procedure

Allow all reagents and samples to reach room temperature and mix thoroughly by gentle inversion before use. Number the microwell plates (Microtiter Strips which are removed from the frame for usage should be marked accordingly to avoid any mix-up). Duplicate determinations are recommended.
The binding of the antisera and of the enzyme conjugate and the activity of the enzyme are temperature dependent. The higher the temperature, the higher the absorption values will be. Varying incubation times will have similar influences on the absorbance. The optimal temperature during the enzyme immunoassay is between 20 – 25 °C.

6.1 Sample preparation

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

- *Using poorly diluted or undiluted samples might lead to incorrect results due to a matrix effect. Therefore, it is advisable to perform a linearity experiment prior to the test. Make different dilutions of a sample with the included diluent (e. g. 1:1, 1:4, 1:10, 1:20 and so on), spike each dilution with the same known concentration and check the recovery. If the samples are found consistently correct and no matrix effect is detected, samples can be used undiluted.*
- *If a matrix effect is detected, samples should be diluted with the included diluent prior to the test. It is also possible to dilute the standards with the sample matrix instead of diluent, in order to create the same matrix conditions for standards and samples. Currently the following buffers/solvents are evaluated for use: Ringer Buffer, PBS and 0.9% NaCl. If another substance is used, please check the compatibility by a Proof of Principle prior to the measurement. Prepare a stock solution of serotonin or use Standard F. Add small amounts (to change the native sample matrix as little as possible) of the stock solution or Standard F to the sample matrix and check the recovery. Please take the correction factor into account (see chapter 7).*
- *If the expected sample concentrations are higher than the standard range (see chapter 4.1) samples should be diluted accordingly with the included diluent. Please take the correction factor into account (see chapter 7).*
- *The measuring range and sensitivity of this test are defined by the correction factor, which is calculated by sample volume and dilution (see chapter 7). If the expected concentrations are unknown, please test different dilutions and amounts of sample volume, to make sure that the samples will fall into the measuring range of this assay.*
- *Serotonin decomposes fast in acidic solution (< pH 3) and at higher temperatures.*
- *When acidic sample solutions are used, protect serotonin by keeping the temperature low (2 – 8 °C). Use pre-cooled buffers and materials. Adjust the pH to (6 – 7.4) as soon as possible.*
- *A pH 7 – 8.5 during acylation (see step 6.3.4) is mandatory.*
- *To protect serotonin against oxidative degradation, add Stabilizer **REF** BA E-5937 (refer to chapter 5).*

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

6.2 Preparation of reagents

Diluent
Dilute the 50 ml Dilution Concentrate **DIL-CONC** **20x** with water to a final volume of 1000 ml.
The Diluent (Diluted Dilution Concentrate) contains *1% Stabilizer **REF** BA E-5937.*

⚠ *The Diluent has to be prepared freshly prior to the assay. Discard after use!*

Wash Buffer
Dilute the 20 ml Wash Buffer Concentrate **WASH-CONC** **50X** with water to a final volume of 1000 ml.
Storage: 2 months at 2 – 8 °C.

Standards and Controls
The Standards and Controls have to be diluted freshly 1 + 1000 with Diluent or buffer*[2] (refer to 6.1), for example: 10 µl Standard + 10 ml Diluent or buffer*[2].
*[2] The buffer used for the respective experiment, enriched with 1% Stabilizer **REF** BA E-5937.*

Serotonin Microtiter Strips
In rare cases, residues of the blocking and stabilizing reagent can be seen in the wells as small, white dots or lines. These residues do not influence the quality of the product.

6.3 Acylation

1.	Pipette **100 µl** of **diluted standards**, **diluted controls,** and **1 – 100 µl of samples** into the respective wells of the **ACYL-PLATE** *[3].
2.	Add **Diluent** or buffer*[2] (refer to 6.1) to the wells containing the samples to a **final volume** of **100 µl.**
3.	Add **25 µl** **ACYL-BUFF** to all wells.
4.	Acylate for **30 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm).
⚠	**100 µl of the acylated standards, controls and samples are needed for the subsequent ELISA.**

*[3] The wells of the Acylation Plate are covered by plastic bars which have to be removed prior to use.

6.4 Serotonin ELISA

1.	Pipette **100 µl** of the **acylated standards, controls and samples** into the appropriate wells of the **Serotonin 5-HIAA Microtiter Strips** **SER** **5-HIAA**.
2.	Pipette **25 µl** of the **SER-AS** into all wells.
3.	Cover plate with **FOILS** and incubate for **15 – 20 h** at **2 – 8 °C**.
4.	Remove the foil. Discard or aspirate the content of the wells. Wash the plate **3 x** by adding **300 µl** of **Wash Buffer**, **discarding** the content and **blotting dry each time** by tapping the inverted plate on absorbent material.
5.	Pipette **100 µl** of the **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 content of the wells. Wash the plate **3 x** by adding **300 µl** of **Wash Buffer**, **discarding** the content and **blotting dry each time** 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 sunlight!***
9.	Add **100 µl** of the **STOP-SOLN** to all wells and shake the microtiter plate shortly.
10.	**Read** the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to **450 nm** (if available a reference wavelength between 620 nm and 650 nm is recommended).

7. Calculation of results

The standard curve, which can be used to determine the concentration of the unknown samples, 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. 4-parameter, marquardt).

⚠ *This assay is a competitive assay. This means: the OD-values are decreasing with increasing concentrations of the analyte. OD-values found below the standard curve correspond to high concentrations of the analyte in the sample and have to be reported as being positive.*

⚠ **The concentrations of the samples taken from the standard curve have to be multiplied by a correction factor:**

$$\text{Correction factor} = \frac{\text{100 µl (volume of standards)}}{\text{sample volume (µl)}}$$

Three different examples are shown to illustrate the calculation of possible correction factors:

Example 1)
10 µl of the undiluted sample are acylated and the concentration taken from the standard curve is 0.02 ng/ml serotonin.
Correction factor = 100/10 = 10
Final concentration of the sample = 0.02 ng/ml x 10 = 0.2 ng/ml serotonin

Example 2)
100 µl of the 1:100 prediluted sample are acylated and the concentration taken from the standard curve is 0.02 ng/ml serotonin.
Correction factor = 100
Final concentration of the sample = 0.02 ng/ml x 100 = 2.0 ng/ml serotonin

Example 3)
10 µl of the 1:100 prediluted sample are acylated and the concentration taken from the standard curve is 0.02 ng/ml serotonin.
Correction factor = 100 x (100/10) = 1000
Final concentration of the sample = 0.02 ng/ml x 1000 = 20 ng/ml serotonin

Conversion
Serotonin [pg/ml] x 5.67 = Serotonin [pmol/l]
Serotonin [pg/sample volume] x 5.67 = Serotonin [pmol/sample volume]

7.1 Quality control

The confidence limits of the kit controls are indicated on the QC-Report.

8. Assay characteristics

8.1 Sensitivity

	Serotonin
Sensitivity	0.005 ng/ml x C*[4]

C*[4] = correction factor (refer to 7.)

8.2 Specificity

Compound	Cross-reactivity (%)
Serotonin	100
Tryptamine	0.19
Melatonin	0.03
5-Hydroxyindole acetic acid	< 0.002
Phenylalanine	< 0.002
Histidine	< 0.002
Tyramine	< 0.002
5-Hydroxytryptophan	< 0.002

⚠ ***For literature or any other information please contact your local supplier.***

⚠ ***The liability of the manufacturer shall be limited to the replacement of defective products. The manufacturer takes no liability for any damages or expenses arising directly or indirectly from the use of this product.***

Symbols:

+8 °C +2	Storage temperature		Manufacturer	Σ	Contains sufficient for <n> tests
	Use-by date	LOT	Batch code		
	Consult instructions for use	CONT	Content		
	Caution	REF	Catalogue number		Distributor
	Date of manufacture		Do not re-use	RUO	For research use only!