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Instructions for use

Serotonin Research ELISA

REF KAPL10-5900

96

+2 +8 °C

RUO For Research use only- Not for use in diagnostic procedures

Serotonin Research ELISA

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.

First, Serotonin is acylated quantitatively. The subsequent competitive ELISA kit uses the microtiter plate format. The antigen is bound to the solid phase of the microtiter plate. The acylated standards, controls and samples and the solid phase bound analyte compete for a fixed number of antiserum binding sites. After the system is in equilibrium, free antigen and free antigen-antiserum 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. The reaction is monitored at 450 nm.
Quantification of unknown samples is achieved by comparing their absorbance with a reference curve prepared with known standard concentrations.

2. Advice on handling the test

2.1 Reliability of the test results

In order to assure a reliable evaluation of the test results it must be conducted according to the instructions included and in accordance with current rules and guidelines (GLP, RILIBÄK, etc.). Special attention must be paid to control checks for precision and correctness during the test; the results of these control checks have to be within the norm range. In case of significant discrepancies between the pre-set assay characteristics of this test and the actual results please contact the manufacturer of the test kit for further instructions.
It is recommended that each laboratory establishes its own reference intervals. The values reported in this test instruction are only indicative.
The results obtained with this test kit should not be taken as the sole reason for any therapeutic consequence but have to be correlated to other diagnostic tests and clinical observations.

2.2 Complaints

In case of complaints please submit to the manufacturer a written report containing all data as to how the test was conducted, the results received and a copy of the original test printout. Please contact the manufacturer to obtain a reclamation form and return it completely filled in to the manufacturer.

2.3 Warranty

This test kit was produced according to the latest developments in technology and subjected to stringent internal and external quality control checks. Any alteration of the test kit or the test procedure as well as the usage of reagents from different charges may have a negative influence on the test results and are therefore not covered by warranty. The manufacturer is not liable for damages incurred in transit.

2.4 Disposal

Residual substances and/or all remaining chemicals, reagents and ready for use solutions, are special refuse. The disposal is subject to the laws and regulations of the federation and the countries. About the removal of special refuse the responsible authorities or refuse disposal enterprises inform. The disposal of the kit must be made according to the national official regulations. Legal basis for the disposal of special refuse is the cycle economic- and waste law.
The appropriate safety data sheets of the individual products are available on the homepage. The safety data sheets correspond to the standard: ISO 11014-1.

2.5 Interference

Do not mix reagents and solutions from different lots. Consider different transport and storage conditions. Inappropriate handling of test samples or deviations from the test regulation can the results affect. Use no kit components beyond the expiration date. Avoid microbiological contamination of the reagents and the washing water. Consider incubation periods and wash references.

2.6 Precautions

Observe the incubation periods and washing instructions. Never pipette by mouth and avoid contact of reagents and specimens with skin. No smoking, eating or drinking in areas where samples or kit test tubes are handled. When working with kit components or samples, always wear protective gloves and wash your hand thoroughly as soon as you have finished the work. Avoid spraying of any kind. Avoid any skin contact with reagents. Use protective clothing and disposable gloves. All steps have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes. Sodium azide could react with lead and copper tubes and may form highly explosive metal azide. When clearing up, rinse thoroughly with large volumes of water to prevent such formation.
All reagents of this testkit which contain human or animal serum or plasma have been tested and confirmed negative for HIV I/II, HbsAg and HCV by FDA approved procedures.
All reagents, however, should be treated as potential biohazards in use and for disposal.

3. Storage and stability

Store the reagents at 2 - 8 °C until expiration date. Do not use components beyond the expiry date indicated on the kit labels. Do not mix various lots of any kit component within an individual assay.

4.1 Contents of the kit

BA R-8901	STANDARD A	**Standard A**	1 x 4 mL	concentrate*
BA R-8902	STANDARD B	**Standard B**	1 x 4 mL	concentrate*
BA R-8903	STANDARD C	**Standard C**	1 x 4 mL	concentrate*
BA R-8904	STANDARD D	**Standard D**	1 x 4 mL	concentrate*
BA R-8905	STANDARD E	**Standard E**	1 x 4 mL	concentrate*
BA R-8906	STANDARD F	**Standard F**	1 x 4 mL	concentrate*
BA R-8951	CONTROL 1	**Control 1**	1 x 4 mL	concentrate*
BA R-8952	CONTROL 2	**Control 2**	1 x 4 mL	concentrate*
BA E-5934	ACYL-PLATE	**Acylation Plate**	1 x 96 wells	ready for use, pre-coated with Acylation Reagent
BA E-5937	ASC-ACID 10%	**Ascorbic Acid**	1 x 4 mL	ready for use
BA E-5941	DIL-CONC 20x	**Dilution Concentrate**	1 x 50 mL	Concentrate. Dilute content with dist. water to a final volume of 1000 mL
BA E-0030	WASH-CONC 50x	**Wash Buffer Concentrate**	1 x 20 mL	Concentrate. Dilute content with dist. water to a final volume of 1000 mL
BA E-0040	CONJUGATE	**Enzyme Conjugate**	1 x 12 mL	ready for use, anti-rabbit IgG conjugated with peroxidase
BA E-0055	SUBSTRATE	**Substrate**	1 x 12 mL	ready for use, containing a solution of tetramethylbenzidine (TMB)
BA E-0080	STOP-SOLN	**Stop Solution**	1 x 12 mL	ready for use, containing 0.25 M sulphuric acid
BA D-0090	FOILS	**Adhesive Foil**	1 x 4	ready for use
BA E-5910	SER-AS	**Serotonin Antiserum**	1 x 6 mL	from rabbit, ready for use, blue coloured, blue screw cap
BA E-5911	ACYL-BUFF	**Acylation Buffer**	1 x 30 mL	ready for use
BA E-0931	TU SER 5-HIAA	**Serotonin/5-HIAA Microtiter Strips**	1 x 96 wells	12 strips, 8 wells each, break apart, pre-coated

⚠ ** Dilute standards and controls 1+ 1000 with **Diluent** or the buffer/solvent, which is used for the experiment, containing 0.1 % (w/v) ascorbic acid.*
Actually the following buffers/solvents are evaluated for use: Ringer Buffer, PBS and 0.9% NaCl.
Other buffers/solvents are suitable but should be evaluated before use!

4.2 Additional materials and equipment required but not provided in the kit

- Calibrated variable precision micropipettes (e.g. 10-100 µL and 100-1000 µl)
- Microtiter plate washing device
- ELISA reader capable of reading absorbance at 450 nm
- Plate shaker (shaking amplitude 3mm; approx. 600 rpm)
- Absorbent material (paper towel)
- Distilled water
- Vortex mixer

5. Sample collection and storage

In general this assay is dedicated for any biological sample such as 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.

⚠ ***To protect Serotonin against oxidative degradation the samples should contain 0.1% (w/v) ascorbic acid.***

6. Test procedure

Allow all reagents and samples to reach room temperature.
Duplicate determinations are recommended.

6.1 Sample preparation

The Serotonin Research 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.

- *At RT (20-25°C) Serotonin is stable at pH 6 – 7.4 enriched with 0.1 % (w/v) ascorbic acid.*
- *The Diluent provided in the kit can be used for dilution of samples.*
- *Serotonin decomposes fast in acidic solution (< pH 3) at RT.*
- *When acidic solutions are used, protect the serotonin by keeping the temperature low (2 -8 °C). Use pre-cooled Buffers and Materials. Adjust the pH to (6 – 7.4).*
- *To protect Serotonin against oxidative degradation add ascorbic acid (refer to 5.).*
- *It is advisable to perform a Proof of Principle to determine the recovery of the serotonin in your samples. Prepare a stock solution of the serotonin. Add small amounts (to change the native sample matrix as less as possible) of the stock solutions to the sample matrix and check the recovery.*
- *Sample volume determines the sensitivity for this test. Test different amounts of sample volume, to see what sample volume is needed to determine serotonin.*

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 with distilled water to a final volume of 1000 mL.
The Diluent (Diluted Dilution Concentrate) contains *0.1 % (w/v) ascorbic acid.*
Storage: up to 12 months 2–8°C

Wash Buffer
Dilute the 20 mL Wash Buffer Concentrate with distilled water to a final volume of 1000 mL.
Storage: up to 6 months 2–8°C

Standards
The standards and controls have to be diluted 1+ 1000 with Diluent or buffer*, for example: 10 µL standard + 10 mL Diluent or buffer*.

* *The buffer used for the respective experiment, enriched with 0.1 % (w/v) ascorbic acid.*

- *The standards have to be prepared freshly prior to the assay.*
- *Evaluated for Ringer Buffer, PBS and 0.9% NaCl. Other buffers can be evaluated quite easily.*
- *A pH of 7 -8.5 during the acylation step is mandatory.*

6.3 Acylation

1.	Pipette **100 µL** of **diluted standards**, **100 µL** of **diluted controls,** and **1 – 100 µL of samples** into the respective wells of the **Acylation Plate**[*)]**.**
2.	Add **Diluent** or buffer* (refer to 6.1) to the wells containing the samples to a **final volume** of **100 µL.**
3.	Add **25 µL Acylation Buffer** to all tubes.
4.	Acylate for **30 minutes** 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

***)** 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.**
2.	Pipette **25 µL** of the **Serotonin Antiserum** into all wells.
3.	Cover plate with **Adhesive Foil** and incubate for **15 -20 hours** at **2 – 8 °C**.
4.	Remove the foil and discard or aspirate the contents of the wells and **wash** each well **3 times** thoroughly with **300 µL Wash Buffer**. Blot dry by tapping the inverted plate on absorbent material.
5.	Pipette **100 µL** of the **Enzyme 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 contents of the wells and **wash** each well **3 times** thoroughly with **300 µL Wash Buffer**. Blot dry 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 sun light!***
9.	Add **100 µL** of the **Stop Solution** to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution.
10.	**Read** the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to **450 nm** and a reference wavelength between 620 nm and 650 nm.

7. Calculation of results

	Concentration of the diluted standards					
Standard	**A**	**B**	**C**	**D**	**E**	**F**
Serotonin (ng/mL)	0	0.015	0.05	0.15	0.5	2.5
Serotonin (nmol/L)	0	0.085	0.28	0.85	2.8	14
Serotonin (pg/sample volume)	0	1.5	5	15	50	250
Serotonin (pmol/ sample volume)	0	8.5	28.4	85	284	1 418
Conversion:	Serotonin (pg/mL) x 5.67 = Serotonin (pmol/L) Serotonin (pg/sample vol.) x 5.67 = Serotonin (pmol/sample vol.)					

The calibration curve from which the concentrations of the samples can be read off, is obtained by plotting the absorbance readings (calculate the mean absorbance) measured for the standards (linear, y-axis) against the corresponding standard concentrations (logarithmic, x-axis).
The use of a non-linear regression for curve fitting (e.g. spline, 4- parameter, akima) is recommended.

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

$$\textbf{Correction factor} = \frac{\textbf{100 µL (volume of standards)}}{\textbf{sample volume (µL)}}$$

Example: 10 µL of the 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

7.1 Quality control
It is recommended to use control samples according to state and federal regulations. Controls with both normal and pathological levels should be used. The kit or other commercially available controls should be found within the established confidence limits. The confidence limits of the kit controls are indicated on the QC-Report.

7.2 Calibration
The binding of the antisera and the enzyme conjugates and the activity of the enzyme used are temperature dependent, and the extinction values may vary if a thermostat is not used. The higher the temperature, the higher the extinction values will be. Corresponding variations also apply to the incubation times. The optimal temperature during the Enzyme Immunoassay is between 20-25°C.

⚠ *In case of overflow, read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to 405 nm*

8. Assay characteristics

8.1 Sensitivity

	Serotonin
Sensitivity	0.005 ng/mL x C*

C* = 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 actual literature, information about clinical significance or any other information please contact your local supplier.***

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code	I V D	For in-vitro diagnostic use only!
	Consult instructions for use	CONT	Content	CE	CE labelled
	Caution	REF	Catalogue number	RUO	For research use only!