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Belgium

Instructions for use

Serotonin RIA

REF KIPL0900

100 +2 +8 °C IVD CE 200 kBq

Serotonin RIA

1. Intended use and principle of the test

^{125}I – Radioimmunoassay for the quantitative determination of Serotonin in serum, urine and platelets.

First, Serotonin is quantitatively acylated.
The assay procedure follows the basic principle of radioimmunoassay, involving competition between a radioactive and a non-radioactive antigen for a fixed number of antibody binding sites. The amount of 125I-labelled antigen bound to the antibody is inversely proportional to the analyte concentration of the sample. When the system is in equilibrium, the antibody bound radioactivity is precipitated with a second antibody in the presence of polyethylene glycol. The precipitate is counted in a gamma counter. Quantification of unknown samples is achieved by comparing their activity with a reference curve prepared with known standards.

2. Storage and stability

The reagents should be stored at 2 - 8 °C until expiration date. Do not use components beyond the expiry date shown on the kit labels.

3. Contents of the kit

BA R-8901	STANDARD A	**Standard A**	1 x 4 mL	ready for use
BA R-8902	STANDARD B	**Standard B**	1 x 4 mL	ready for use
BA R-8903	STANDARD C	**Standard C**	1 x 4 mL	ready for use
BA R-8904	STANDARD D	**Standard D**	1 x 4 mL	ready for use
BA R-8905	STANDARD E	**Standard E**	1 x 4 mL	ready for use
BA R-8906	STANDARD F	**Standard F**	1 x 4 mL	ready for use
BA R-8910	AS SER	**Serotonin Antiserum**	1 x 5.25 mL	from rabbit, ready for use, blue coloured, blue screw cap
BA R-8911	ACYL-BUFF	**Acylation Buffer**	1 x 30 mL	ready for use
BA R-8912	ACYL-REAG	**Acylation Reagent**	1 x 3 mL	ready for use
BA R-0920	^{125}I-SER	**^{125}I – Serotonin**	1 x 5.5 mL	activity < 200 kBq, ready for use, red coloured, red screw cap
BA R-8951	CONTROL 1	**Control 1**	1 x 4 mL	ready for use
BA R-8952	CONTROL 2	**Control 2**	1 x 4 mL	ready for use
BA R-0025	PREC-REAG	**Precipitating Reagent**	1 x 55 mL	ready for use, goat anti-rabbit serum in PEG phosphate buffer. *Mix thoroughly before use!*

4. Additional materials and equipment required but not provided with the kit

- Calibrated variable precision micropipettes (e.g. 10-100 µL / 100-1000 µL)
- Plastic tubes (polypropylene, polystyrene) and suitable rack
- Centrifuge (preferable refrigerated) capable of at least 3,000 x g
- Suitable device for aspirating or decanting the tubes.
- Vortex mixer
- Gamma counter
- Distilled water

5. Sample collection and storage

Serum
Haemolytic and especially lipemic samples should not be used for the assay.
Storage: up to 24 hours at 2 - 8°C, for longer period (up to 6 months) at - 20°C.
Repeated freezing and thawing should be avoided.

Urine
Spontaneous or 24-hour urine, collected in a bottle containing 10-15 mL of 6 M HCl, as well may be used.
Storage: for a longer period (up to 6 months) at -20°C. Avoid exposure to direct sunlight.
Repeated freezing and thawing of the samples should be avoided.

Plasma
More than 98 percent of the circulating serotonin is located in the platelets and is released during blood clotting. Blood has to be collected by venipuncture into plastic tubes containing EDTA or Citrate.

Platelet-rich plasma (PRP)
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.

Platelets
A 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). Discard the supernatant.
200 µL of dist. water is added to the pellet and mixed thoroughly on a vortex mixer. This suspension can then 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.
25 µL of the supernatants are used for the acylation reaction.

Cerebrospinal fluid (CSF)
Storage: at - 20 °C.

Platelet-free plasma (PFP)
To measure serotonin in **platelet-free plasma (PFP)**, an aliquot of the **PRP** is centrifuged at 4,500 x g for 10 min. at 4°C. Platelet-free plasma can be stored at –20°C for up to two weeks.

100 µL of the supernatant is used for the acylation reaction.

6. Test procedure

Allow all reagents – with the exception of Precipitating Reagent - to reach room temperature and mix thoroughly by gentle inversion before use. Number the assay tubes **(polystyrene or polypropylene)** accordingly. Duplicate determinations are recommended.

⚠ *Pipetted liquids should not adhere to the wall of the RIA tubes. If necessary please centrifuge tubes for 1 minute at 500xg to spin down adhering liquids.*

⚠ *Do not use glass tubes for the assay!*

6.1 Sample preparation and acylation

6.1.1 Serum, urine and platelets

1.	Pipette **25 µL** of **standards**, **25 µL** of **controls,** and **25 µL** of **serum, urine** and **platelets** into the respective tubes.
2.	Add **250 µL Acylation Buffer** to all tubes.
3.	Add **25 µL** of **Acylation Reagent** to all tubes.
4.	Mix thoroughly and incubate for **30 minutes** at **RT** (20-25°C).
5.	Pipette **2 mL** of **distilled water** into all tubes and mix thoroughly.
⚠	Take **25 µL** of the acylated standards, controls and samples for the Serotonin RIA

6.1.2 Cerebrospinal fluid and platelet-free plasma

1.	Pipette **25 µL** of **standards** and **controls, 100 µL** of **cerebrospinal fluid (CSF)** and **platelet-free plasma (PFP)** into the respective tubes.
2.	Pipette **250 µL Acylation Buffer** into the tubes for **standards** and **controls** and **50 µL** into the tubes for **CSF** and **PFP**.
3.	Pipette **25 µL** of **Acylation Reagent** into the tubes for **standards** and **controls** and **5 µL** into the tubes for **CSF** and **PFP**.
4.	Mix thoroughly and incubate for **30 minutes** at **RT** (20-25°C).
5.	Pipette **2 mL** of **distilled water** into the tubes for **standards** and **controls** and **300 µL** into the tubes for **CSF** and **PFP**.
⚠	Take **25 µL** of the acylated standards, controls and samples for the Serotonin RIA

6.2 Serotonin RIA

1.	Pipette **25 µL** of **prepared Standard A** into the tubes for the **NSB**.
2.	Pipette **25 µL** of **prepared standards, controls** and **samples** into the respective tubes.
3.	Pipette **50 µL** of the **^{125}I Serotonin** into **all tubes**.
4.	Pipette **50 µL** of **Serotonin Antiserum** into **all tubes** (***except totals and NSB***); mix thoroughly.
5.	Cover tubes. Incubate for **90 minutes** at **2 - 8 °C.**
6.	Mix the chilled (2 - 8 °C) **Precipitating Reagent** thoroughly, pipette each **500 µL** into **all tubes** (***except totals***), and mix on a vortex.
7.	Incubate for **15 minutes** at **2 - 8 °C**.
8.	Centrifuge for **15 minutes** at **3,000 x g**, if possible in a refrigerated centrifuge.
9.	**Decant** or aspirate the **supernatant <u>carefully</u>** (***except totals***). Blot the tubes dry and leave them upside for 2 minutes.
10.	**Count** all tubes for **1 minute** in a gamma counter.

7. <u>Calculation of results</u>

	Concentration of the standards					
Standard	**A**	**B**	**C**	**D**	**E**	**F**
Serotonin (ng/mL)	0	15	50	150	500	2,500
Serotonin (nmol/L)	0	85.1	284	851	2,840	14,175
Conversion:	Serotonin (ng/mL) x 5.67 = Serotonin (nmol/L)					

Subtract the mean cpm of the non-specific binding NSB from the mean cpm of standards, controls and samples.
The calibration curve from which the concentrations in the samples can be read off, is obtained by plotting the percentage of (B-NSB)/(B0-NSB) measured for the standards (linear, y-axis) against the corresponding standard concentrations (logarithmic, x-axis).
Use a non-linear regression for curve fitting (e.g. spline, 4- parameter, akima).

The concentrations for serum, urine and platelets can be read directly from the standard curve, while the read concentrations for the platelet-free plasma and the cerebrospinal fluid have to be divided by 20.

7.1 Calculation of serotonin in platelets

The content of serotonin in platelets is referred to 10^9 platelets.
Example:
Measured Serotonin concentration: 100 ng/mL
Number of the platelets in the PRP: 300.000 / µL = 0,3 x 10^9 platelets/mL with a serotonin content of 100 ng.
The resulting serotonin content in the platelets is 333 ng/ 10^9 platelets.
(100 ng serotonin x 1.0 x 10^9 /0.3 x 10^9)

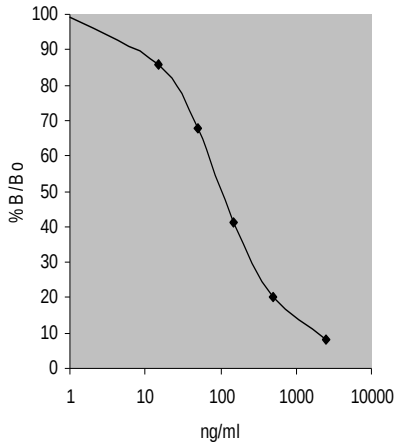
7.2 Quality control

It is recommended to use control samples according to state and federal regulations. Use controls at both normal and pathological levels. The kit or other commercially available, controls should fall within established confidence limits. The confidence limits of the kit controls are indicated on the QC-report.

7.3 Typical calibration curve

⚠ *Example, do not use for calculation!*

SEROTONIN



8. Assay characteristics

Expected Reference Values		**Serotonin**	
	Serum	♀ 80 - 450 ng/mL	♂ 40 - 400 ng/mL
	Urine	50 - 250 µg/day	
	Platelet-free Plasma (PFP)	1.8 – 7.5 ng/mL	
	Serotonin in platelets	215– 850 ng/10^9 platelets	

Analytical Sensitivity (Limit of Detection)	**Serotonin Serum, urine and platelets**	**Serotonin Cerebrospinal fluid and platelet-free plasma**
	6.7 ng/mL	0.3 ng/mL

Analytical Specificity (Cross Reactivity)	**Substance**	**Cross Reactivity (%)**
		Serotonin
	Serotonin	100
	Tryptamine	3.000
	Melatonin	0.056
	5-Hydroxyindole acetic acid	0.002
	5-Hydroxy-2-carboxylic acid	<0.001
	Phenylalanine	<0.001
	Histidine	<0.001
	Tyramine	<0.001
	5-Hydroxytryptophan	<0.001
	Tyrosine	<0.001

Precision

Intra-Assay				**Inter-Assay**			
	Sample	Range (ng/mL)	CV (%)		Sample	Range (ng/mL)	CV (%)
Serotonin	1	109 ± 5.1	4.74	Serotonin	1	96 ± 5.6	5.6
	2	253 ± 11	4.18		2	301 ± 14	4.6

Linearity			Range	Serial dilution up to	Range (%)
	Serotonin	Urine	55 – 1,029 ng/mL	1:16	89 – 116
		Serum	55 – 1,029 ng/mL	1:16	87 – 110

Recovery			Mean (%)	Range (%)	% Recovery after spiking
	Serotonin	Urine	94	85-105	
		Serum	98	82-112	

Method Comparison versus ELISA*	Serotonin	ELISA = 1.26 RIA – 20.53	r = 0.99; n = 37

***** ELISA Immunotech

9. Advice on handling the test

9.1 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.

9.2 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 complaint form and return it completely filled in to the manufacturer.

9.3 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.

9.4 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.

9.5 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.

9.6 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.

This kit contains ^{125}Iodine (half life: 60 days), emitting ionizing X- (28 kev) and G- (35.5 kev) radiations.

The radioactive material may be received, acquired, possessed, and used only by physicians, veterinarians in the practice of veterinary medicine, clinical laboratories or hospitals and only for in vitro clinical or laboratory tests not involving internal or external administration of the material, or the radiation there from, to human beings or animals. Its receipt, acquisition, possession, use, and transfer are subject to the regulations and a general license of the U.S. Nuclear Regulatory Commission or of a State with which the Commission has entered into an agreement for the exercise of regulatory authority. In no case the product must be administered to humans or animals.

All radioactive handling should be executed in a designated area, away from regular passage. A log book for receipt and storage of radioactive materials must be kept in the lab. Laboratory equipment and glassware, which could be contaminated with radioactive substances, should be segregated to prevent cross contamination of different radioisotopes.

Any radioactive spills must be cleaned immediately in accordance with the radio safety procedures. The radioactive waste must be disposed of following the local regulations and guidelines of the authorities holding jurisdiction over the laboratory. Adherence to the basic rules of radiation safety provides adequate protection.

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.

⚠ ***Actual literature, information about clinical significance or any other information about the test are available on the homepage or contact the manufacturer directly.***

Symbols:

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