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Instructions for use

Serotonin RIA

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

For Research Use Only – Not for Use in Diagnostic Procedures.

REF **IB88189**

Σ 100

+2 +8 °C

RUO

200 kBq

1. Introduction

1.1 Intended use and principle of the test

^{125}I – Radioimmunoassay for the quantitative determination of Serotonin in serum, urine, and platelets. For Research Use Only – Not For Use in Diagnostic Procedures.

This kit can also be used for cerebrospinal fluid (CSF) and platelet-free plasma (PFP). Please refer to appendix 1.

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. When the system is in equilibrium, the antibody bound radioactivity is precipitated with a second antibody in the presence of polyethylene glycol. After centrifugation and decantation of the supernatant the precipitate is counted in a gamma counter. The amount of ^{125}I-labelled antigen bound to the antibody is inversely proportional to the analyte concentration of the sample. Quantification of unknown samples is achieved by comparing their activity with a reference curve prepared with known standards.

1.2 Background

Serotonin (5-hydroxytryptamine) is an intermediate product of tryptophan metabolism and is located primarily in the enterochromaffin cells of intestine (EC-cells), serotonergic neurons of the brain, platelets of the blood and is well established as a neurotransmitter in the central nervous system. EC-cell production accounts for 80% of the body's serotonin content. Serotonin is predominately metabolized to 5-hydroxyindoleacetic acid (5-HIAA), which is excreted by the kidneys.

2. Procedural cautions, guidelines, warnings and limitations

2.1 Precautions, 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 has to be used to run the assay. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
(2) This assay was validated for a certain type of sample as indicated in *Intended Use* (please refer to Chapter 1). Any off-label use of this kit is in the responsibility of the user and the manufacturer cannot be held liable.
(3) The principles of Good Laboratory Practice (GLP) have to be followed.
(4) In order to reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves and protective glasses where necessary.
(5) All kit reagents - with the exception of Precipitating Reagent – and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
(6) For the dilution or reconstitution purposes use deionized, distilled, or ultra-pure water.
(7) The radioactive material (^{125}Iodine, half life 60 days, emitting ionizing X-radiation with 28 keV and G-radiation with 35.5 keV) may be received, acquired, possessed and used only by physicians, laboratories or hospitals. In compliance with regulations, a copy of the customer's current radioisotope license must be on file with the supplier. Orders cannot be shipped until the license is received by the supplier (Radiation Protection Act of June 30, 1989).
(8) Duplicate determination of sample is highly recommended to be able to identify potential pipetting errors.
(9) Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials and devices are prepared ready at the appropriate time.
(10) Incubation times do influence the results. All tubes should be handled in the same order and time intervals.
(11) To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
(12) A standard curve must be established for each run.
(13) The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report.
(14) 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.
(15) For information on hazardous substances included in the kit please refer to Material Safety Data Sheet (MSDS). The Material Safety Data Sheet for this product is available directly on the website of the manufacturer or upon request.
(16) The expected reference values reported in this test instruction are only indicative. It is recommended that each laboratory establishes its own reference intervals.
(17) 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.
(18) Kit reagents must be regarded as hazardous waste and disposed according to national regulations.

2.2 Limitations

Any inappropriate handling of samples or modification of this test might influence the results.

2.2.1 Interfering substances

Serum/Plasma

Samples containing precipitates or fibrin strands or which are haemolytic or lipemic might cause inaccurate results.

24-hour urine

Please note the sample preparation! If the percentage of the final concentration of acid is too high, this will lead to incorrect results for the urine samples.

2.2.2 Drug interferences

There are no known substances (drugs) which ingestion interferes with the measurement of Serotonin level in the sample.

2.2.3 High-Dose-Hook effect

No hook effect was observed in this test.

3. Storage and stability

Store the unopened reagents at 2 - 8 °C until expiration date. Do not use components beyond the expiry date indicated on the kit labels. Once opened the reagents are stable for 1 month when stored at 2 – 8 °C.

4. Materials

4.1 Content of the kit

BA R-8910 SER-AS **Serotonin Antiserum** - Ready to use

Content: Rabbit anti-serotonin antibody, blue coloured

Volume: 1 x 5.25 ml/vial, blue cap

BA R-0920 ^{125}I-SER **^{125}I – Serotonin** - Ready to use

Content: ^{125}I labeled Serotonin, red coloured

Volume: 1 x 5.5 ml/vial, orange cap

Hazards identification:

Radioactive, activity < 200 kBq

BA R-8912 ACYL-REAG **Acylation Reagent** - Ready to use

Content: Acylation reagent in dimethylsulfoxide and dimethylformamide

Volume: 1 x 3 ml/vial, green cap

Hazards identification:

H225 Highly flammable liquid and vapour.
H360 May damage fertility or the unborn child.
H319 Causes serious eye irritation.

BA R-8911 ACYL-BUFF **Acylation Buffer** - Ready to use

Content: TRIS buffer with non-mercury preservative

Volume: 1 x 30 ml/vial, light grey cap

Standards and **Controls** - Ready to use

Cat. no.	Component	Colour/Cap	Concentration ng/ml	Concentration nmol/l	Volume/ Vial
BA R-8901	STANDARD A	white	0	0	4 ml
BA R-8902	STANDARD B	light yellow	15	85.1	4 ml
BA R-8903	STANDARD C	orange	50	284	4 ml
BA R-8904	STANDARD D	dark blue	150	851	4 ml
BA R-8905	STANDARD E	light grey	500	2 835	4 ml
BA R-8906	STANDARD F	black	2 500	14 175	4 ml
BA R-8951	CONTROL 1	light green	Refer to QC-Report for expected value and acceptable range!		4 ml
BA R-8952	CONTROL 2	dark red			4 ml

Conversion: Serotonin (ng/ml) x 5.67 = Serotonin (nmol/l)

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

BA R-0025 PREC-REAG **Precipitating Reagent -** Ready to use

Content: Goat anti-rabbit serum in PEG phosphate buffer

Volume: 1 x 55 ml/vial, white cap

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

- Calibrated precision pipettes to dispense volumes between 25 - 2000 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
- Water (deionized, distilled, or ultra-pure)
- Absorbent paper (paper towel)

5. Sample collection and storage

Foods or liquids containing serotonin such as pineapple, eggplant, avocados, bananas, currants, kiwis, melon, mirabelles, plums, peaches chocolate, gooseberries, tomatoes, or walnuts, should be avoided 2 days before and including the day of the sample collection (24-hour urine). Selective Serotonin Reuptake Inhibitors (SSRIs) influence serotonin levels. People who are taking such medications should consult with their doctor before specimen collection.
Repeated freezing and thawing of the samples should be avoided.

Serum
Collect blood by venipuncture (Monovette™ or Vacuette™ for serum), allow to clot, and separate serum by centrifugation (according to manufacturer's instructions). Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.
Haemolytic and lipemic samples should not be used for the assay.
Storage: up to 24 hours at 2 - 8 °C, for longer period (up to 6 month) at -20 °C.

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.
Storage: for longer periods (up to 6 months) at -20 °C.
Avoid exposure to direct sunlight.

Platelets
More than 98 percent of the circulating serotonin is located in the platelets and is released during blood clotting. Blood must be collected by venipuncture in plastic tubes (Monovette™ or Vacuette™) 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.
25 µl of the supernatant is used for the acylation reaction.

6. Test procedure

For research use only (RUO) this kit can also be used for cerebrospinal fluid (CSF) and platelet-free plasma (PFP). Please refer to appendix 1.

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 500 x g to spin down adhering liquids. Do not use glass tubes for the assay!*

6.1 Sample preparation and acylation for serum, urine and platelets

1.	Pipette **25 µl** of **standards**, **controls**, **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 min** at **RT** (20 - 25 °C).
5.	Pipette **2 ml** of **water** (deionized, distilled, or ultra-pure) into **all tubes** and mix thoroughly (vortex).
⚠	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 (vortex).
5.	Cover **tubes**. Incubate for **90 min** 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 min** at **2 - 8 °C**.
8.	Centrifuge for **15 min** at **3,000 x g**, if possible in a refrigerated centrifuge.
9.	**Decant** or aspirate the **supernatant carefully *(except totals)***. Blot the tubes dry and leave them upside down for 2 minutes.
10.	**Count** all tubes for **1 min** in a gamma counter.

7. Calculation of results

Measuring range	**Serotonin**
	6.7 – 2 500 ng/ml

Subtract the mean cpm of the non-specific binding NSB from the mean cpm of standards, controls and samples.
The standard curve from which the concentrations in the samples can be read off, is obtained by plotting the percentage of (B-NSB)/ (B_0-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).

⚠ *This assay is a competitive assay. This means: the counts are decreasing with increasing concentrations of the analyte. Counts 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 for **serum**, **urine**, **platelets** and **controls** can be read directly from the standard curve.

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)

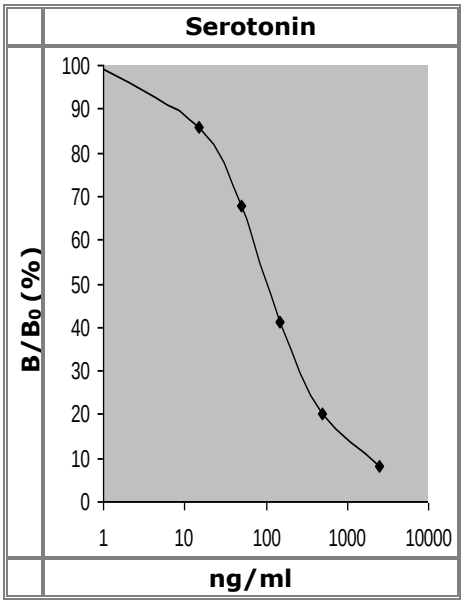
Conversion
Serotonin (ng/ml) x 5.67 = Serotonin (nmol/l)

Expected reference values
It is strongly recommended that each laboratory should determine its own reference values.

	Serotonin
Serum	70 – 270 ng/ml
24-hour urine	50 - 250 µg/24h
Serotonin in platelets	500 - 950 ng/10^9 platelets

7.1 Quality control
It is recommended to use control samples according to national 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.

7.2 Typical standard curve
⚠ *Example, do not use for calculation!*



8. Assay characteristics

Sensitivity	Limit of Detection (LOD) Limit of Quantitation (LOQ)	6.6 ng/ml 10.5 ng/ml

	Substance	Cross Reactivity (%)
		Serotonin
Analytical Specificity (Cross Reactivity)	Serotonin	100
	Tryptamine	3.64
	Melatonin	0.06
	5-Hydroxyindole acetic acid	<0.01
	5-Hydroxy-2-carboxylic acid	<0.01
	Phenylalanine	<0.01
	Histidine	<0.01
	Tyramine	<0.01
	5-Hydroxytryptophan	<0.01
	Tyrosine	<0.01

Precision							
Intra-Assay				**Inter-Assay**			
	Sample	Range (ng/ml)	CV (%)		Sample	Range (ng/ml)	CV (%)
Urine	1	106 ± 12.4	11.7	Urine	1	128 ± 16.3	6.8
	2	401 ± 44.0	11.0		2	174 ± 15.8	7.9
	3	1373 ± 100	7.3		3	358 ± 26.1	5.9
Serum	1	253 ± 10.5	4.2	Serum	1	86.7 ± 5.9	12.8
	2	164 ± 9.7	5.9		2	144 ± 11.4	9.1
					3	363 ± 21.5	7.3

			Range	Serial dilution up to	Range (%)
Linearity	Serotonin	Urine	55 – 1 029 ng/ml	1:21	89 – 116
		Serum	48 – 981 ng/ml	1:21	81 – 102

			Mean (%)	Range (%)	% Recovery after spiking
Recovery	Serotonin	Urine	88	83 – 94	
		Serum	98	90 – 107	

Method Comparison versus ELISA*	Urine	ELISA = 1.49 RIA – 44.03	R^2 = 0.99; n = 19
* ELISA Immunotech	Serum	ELISA = 1.25 RIA – 20.09	R^2 = 0.99; n = 20

9. References/Literature

(1) Peng et al. Role of 5-hydroxytryptamine expression in cerebellar Purkinje cells in obstructive sleep apnea syndrome. Neural Regeneration Research, 7(8):606-610 (2012)

(2) Wozniak et al. Serotonin and Neuron-specific Enolase: Serum Acute Mid-term Levels and their Association With Posttraumatic Depression. Neurosurgery Quarterly, 20(4):297-303 (2010)

(3) Tan et al. Circadian rhythm of salivary serotonin in patients with major depressive disorder Neuroendocrinology Letters 28(4):101-106 (2007)

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⚠ ***For updated literature 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!

APPENDIX 1: – For research use only (RUO) –

Protocol for the determination of Serotonin in **cerebrospinal fluid (CSF) and platelet-free plasma (PFP) – For research use only, not for use in diagnostic procedures.**

1. Sample collection and storage

Platelet-free plasma (PFP)
First, a platelet-rich plasma (PRP) is prepared by centrifugation of plasma (EDTA or citrate) for 10 minutes at room temperature (200 x g) and then the supernatant is transferred to another tube. To measure serotonin in **platelet-free plasma (PFP)**, an aliquot of the supernatant **(PRP)** is centrifuged at 4,500 x g for 10 minutes at 4 °C. This platelet-free plasma can be stored at -20 °C for up to two weeks.

Cerebrospinal fluid (CSF)
CSF should be stored at -20 °C.

2. 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 500 x g to spin down adhering liquids. Do not use glass tubes for the assay!*

2.1 Sample preparation and acylation

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 min** at **RT** (20 - 25 °C).
5.	Pipette **2 ml** of **water** (deionized, distilled, or ultra-pure) 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

2.2 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 min** 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 min** at **2 - 8 °C**.
8.	Centrifuge for **15 min** at **3,000 x g**, if possible in a refrigerated centrifuge.
9.	**Decant** or aspirate the **supernatant carefully *(except totals)***. Blot the tubes dry and leave them upside down for 2 minutes.
10.	**Count** all tubes for **1 minute** in a gamma counter.

3. Calculation of results

Measuring range for CSF and PFP samples	Serotonin
	0.75 – 125 ng/ml

Subtract the mean cpm of the non-specific binding NSB from the mean cpm of standards, controls and samples.
The standard curve from which the concentrations in the samples can be read off, is obtained by plotting the percentage of (B-NSB)/ (B_0-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).

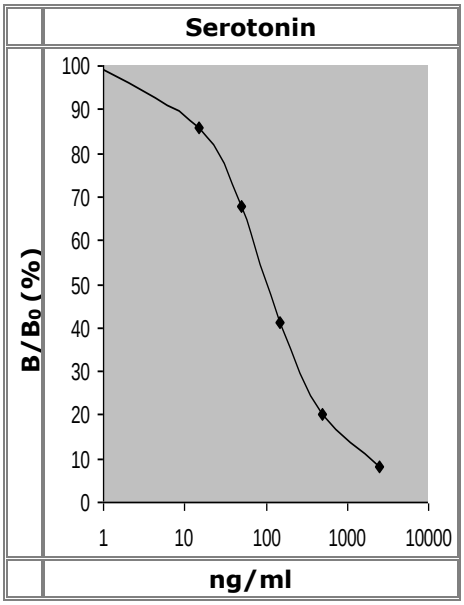
⚠ *This assay is a competitive assay. This means: the counts are decreasing with increasing concentrations of the analyte. Counts found below the standard curve correspond to high concentrations of the analyte in the sample and have to be reported as being positive.*

The read concentrations for the **platelet-free plasma and the cerebrospinal fluid** have to be **divided by 20**.

Conversion
Serotonin (ng/ml) x 5.67 = Serotonin (nmol/l)

Typical standard curve

⚠ *Example, do not use for calculation!*



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