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Instructions for use

Serotonin RIA Fast Track

REF **BA R-8900**

IVD CE 200 kBq

Serotonin RIA

1. Intended use and principle of the test

^{125}I – Radioimmunoassay for the in-vitro diagnostic quantitative determination of Serotonin in serum, urine, and platelets.
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 starting on page 7.

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 ^{125}I-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. Precautions, Guidelines and Warnings

- 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.
- Reagents of this kit which contain human 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.
- The principles of Good Laboratory Practice (GLP) have to be followed.
- In order to reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves and protective glasses where necessary.
- 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).
- For the dilution or reconstitution purposes use deionized, distilled, or ultra-pure water.
- Duplicate determination of sample is highly recommended to be able to identify potential pipetting errors.
- 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.
- Incubation times do influence the results. All tubes should be handled in the same order and time intervals.
- To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
- A calibrator curve must be established for each run.
- The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report.
- 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.
- Some reagents contain sodium azide (NaN_3) as preservatives. In case of contact with eyes or skin, rinse off immediately with water. NaN_3 may react with lead and copper plumbing to form explosive metal azides. When disposing reagents, flush with a large volume of water to avoid azide build-up.
- For information on hazardous substances included in the kit please refer to Material Safety Data Sheets (MSDS). The Material Safety Data Sheet for this product is available directly on the website of the manufacturer or upon request.
- The expected reference values reported in this test instruction are only indicative. It is recommended that each laboratory establishes its own reference intervals.

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. Materials

4.1 Contents of the kit

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

4.2 Additional materials and equipment required but not provided with 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)

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.

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, 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.

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.

Add 200 µl of water (deionized, distilled, or ultra-pure) 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.

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 starting on page 7.

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.
⚠	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 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 for 2 minutes.
10.	**Count** all tubes for **1 min** in a gamma counter.

7. Calculation of results

	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**, **platelets** and **controls** can be read directly from the standard curve.

7.1 Calculation of serotonin in platelets

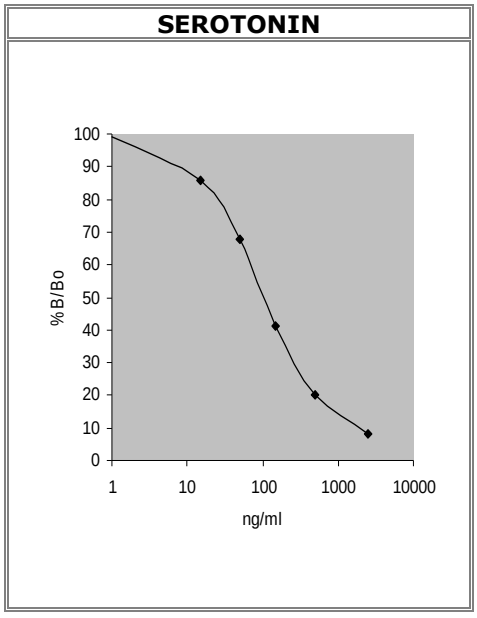
The content of serotonin in platelets is referred to 10^9 platelets.
Example:
Measured Serotonin concentration: 100 ng/ml
Number of the platelets in the PRP: 300.000 / µl = 0.3 x 10^9 platelets/ml with a serotonin content of 100 ng.
The resulting serotonin content in the platelets is 333 ng/ 10^9 platelets.
(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 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.

7.3 Typical calibration curve

⚠ *Example, do not use for calculation!*



8. Assay characteristics

Expected Reference Values		**Serotonin**
	Serum	70 – 270 ng/ml
	Urine	50 - 250 µg/24h
	Platelets	500 – 950 ng/10^9 platelets

Analytical Sensitivity (Limit of Detection)	**Serum, urine and platelets**
	6.7 ng/ml

	Substance	**Cross Reactivity (%)**
		Serotonin
Analytical Specificity (Cross Reactivity)	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

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

			Mean (%)	Range (%)	% Recovery after spiking
Recovery	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

⚠ ***For updated 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!

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

Protocol for the quantitative determination of Serotonin in **cerebrospinal fluid (CSF) and platelet-free plasma (PFP)**

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 for 2 minutes.
10.	**Count** all tubes for **1 minute** in a gamma counter.

3. Calculation of results

	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)					

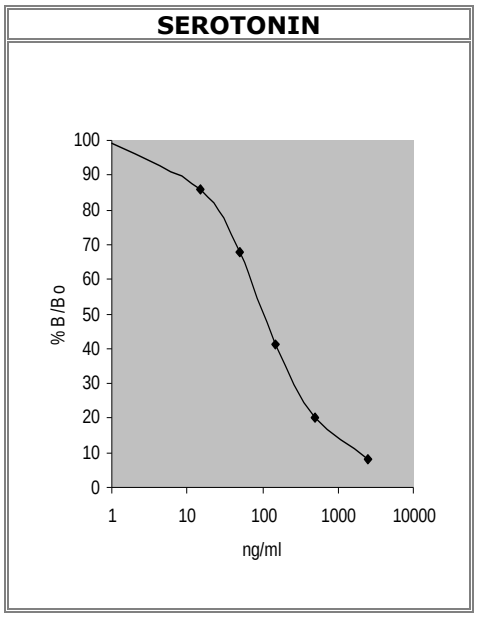
Subtract the mean cpm of the non-specific binding NSB from the mean cpm of standards, controls and samples.

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

Typical calibration curve

⚠ *Example, do not use for calculation!*



4. Assay characteristics

Analytical Sensitivity (Limit of Detection)	**Cerebrospinal fluid and platelet-free plasma**
	0.3 ng/ml