DRG® Serotonin ELISA (EIA-1300)

Revised 22 June 2012 rm (Vers. 11.1) **IVD**

Please use only the valid version of the package insert provided with the kit.

INTENDED USE

Enzyme immunoassay for the *in-vitro diagnostic* quantitative determination of Serotonin in human serum, plasma, platelets and urine.
Further the test can be used for research of tissue homogenates and cell culture supernatants.

SUMMARY AND EXPLANATION

Serotonin is an intermediate product of tryptophan metabolism and is located primarily in the enterochromaffin cells of intestine, serotonergic neurons of the brain, platelets of the blood and is well established as a neurotransmitter in the central nervous system.
Nearly all of the serotonin in circulating blood is concentrated in platelets. Altered concentrations of circulating serotonin have been implicated in several pathological conditions including chronic tension headache, schizophrenia, hypertension, Huntington´s disease, Duchenne´s muscular dystrophy and early acute appendicitis. The determination of serum serotonin levels is of high clinical significance for diagnostic assessment of carcinoid syndrome. An increasing interest in the determination of serotonin in platelets including uptake and release kinetics is expected in the near future.

TEST PRINCIPLE

The sample preparation (derivatization of serotonin to N-acylserotonin) is part of the sample dilution and is achieved by incubation of the respective sample with the Acylation Reagent.
The assay procedure follows the basic principle of competitive ELISA whereby there is competition between a biotinylated and a non-biotinylated antigen for a fixed number of antibody binding sites. The amount of biotinylated antigen bound to the antibody is inversely proportional to the analyte concentration of the sample. When the system is in equilibrium, the free biotinylated antigen is removed by a washing step and the antibody bound biotinylated antigen is determined by use of streptavidine alkaline phosphatase as marker and p-nitrophenyl phosphate as substrate.
Quantification of unknowns is achieved by comparing the enzymatic activity of unknowns with a response curve prepared by using known standards.

WARNINGS AND PRECAUTIONS

1. For in-vitro diagnostic use only. For professional use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. In case of severe damage of the kit package please contact DRG or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
4. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
6. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details. Material Safety Data Sheets for this product are available upon request

7. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
8. Avoid contact with Stop solution. It may cause skin irritations and burns.

STORAGE AND STABILITY

The kit is shipped at ambient temperature and should be stored at 2 °C - 8 °C. Keep away from heat or direct sun light. The storage and stability of specimen and prepared reagents is stated in the corresponding chapters.

The microtiter strips are stable up to the expiry date of the kit in the broken, but tightly closed bag when stored at 2 °C - 8 °C.

SPECIMEN COLLECTION AND STORAGE

⚠ Certain foods contain substantial amounts of serotonin. Furthermore some medications may cause the release of serotonin and may lead to altered levels. Patients have to be abstained from such serotonin rich food (e.g. avocados, bananas, coffee, plums, pineapple, tomatoes, walnuts) as well as some medications (e.g. aspirin, corticotropin, MAO inhibitors, phenazetin, catecholamines, reserpin, nicotin).

Serum

The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Do not use grossly hemolytic, icteric or grossly lipemic specimens. Samples appearing turbid should be centrifuged before testing to remove any particulate material.

Storage:	18 °C -25 °C	2 °C - 8 °C	≤ -20°C (Aliquots)	Keep away from heat or direct sun light.
Stability	2 h	6 h	3 month	Avoid repeated freeze-thaw cycles. Ship samples frozen.

Urine

It is possible to use spontaneous as well as 24 h urine. The total volume of urine excreted during a 24 h period should be collected and mixed in a single bottle containing 10 - 15 mL of 6 N HCl as preservative. Determine total volume for calculation of results. **Mix and centrifuge samples before use in the assay.**

Storage:	≤ -20 °C (Aliquots)	Keep away from heat or direct sun light.
Stability	6 month	Avoid repeated freeze-thaw cycles.

Plasma, 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 into plastic tubes containing EDTA or Citrate as anticoagulant (e.g. 10 mL Monovette NC with 1 mL Citrate solution from SARSTEDT).

Samples are kept and centrifuged at room temperature for 10 min at 200 x g to obtain **platelet-rich plasma (PRP)**. The PRP-supernatant is then transferred to another tube and the platelets counted.

To obtain the **platelet pellet**, an aliquot of 200 µL of PRP (containing between 350000 and 500000 platelets/µL) is added to 800 µL of physiological saline and centrifuged at 4500 x g for 10 min at 4°C (or at 10 000 x g for 2 min at 4 °C). The supernatant is then discarded.

200 µL of double-distilled water is added to the pellet, which can then be stored frozen at < -20 °C for several weeks without any alteration of serotonin content.

CE

Revised 22 June 2012 rm (Vers. 11.1) **IVD**

After thawing of the frozen samples centrifuge at 10 000 x g for 2 min at room temperature. **20 µL of the supernatant** are used in the ELISA (see Acylation).

If you want to measure serotonin in **platelet-free plasma (PFP)**, an aliquot of the PRP is centrifuged at 4500 x g for 10 min at 4°C (or at 10000 x g for 2 min at 4 °C) to obtain platelet-free plasma (PFP). **50 µL of the supernatant** are used in the ELISA for the measurement of the free (not bound to platelets) serotonin (see Acylation).

NOTE: The direct determination of serotonin in PRP has shown that in about 10 % of the PRP samples unpredictable high serotonin concentrations were measured (results obtained by HPLC and Fluorometry). To avoid such discrepancies, the separate measurement of serotonin in platelets and platelet-free plasma is recommended.

	Platelet-free Plasma	**Platelets (after separation from plasma)**		Keep away from heat or direct sun light. Avoid repeated freeze-thaw cycles. Ship samples frozen.
Storage:	≤ -20 °C (Aliquots)	≤ -20 °C (Aliquots)	≤ -80 °C (Aliquots)	
Stability:	2 weeks	4 weeks	12 month	

Tissue homogenates, Cell Culture Supernatants

Centrifuged tissue homogenates and cell culture supernates may be used without special precautions. Caution: Cell culture media may contain serotonin!

Storage:	≤ -20 °C (Aliquots)	Keep away from heat or direct sun light Avoid repeated freeze-thaw cycles.
Stability:	6 month	

MATERIALS SUPPLIED

⚠ The reagents provided with this kit are sufficient for single determinations in the sample preparation (acylation) and duplicates in the assay. Additional reagents are available upon request.

Quantity	**Symbol**	**Component**
1 x 12 x 8	**MTP**	**Microtiter Plate** Break apart strips. Coated with anti-rabbit antiserum (goat).
1 x 7 mL	**ANTISERUM**	**Serotonin Antiserum** Blue colored. Ready to use. Contains: Antiserum (rabbit), phosphate buffer, < 0.1 % NaN_3.
1 x 5 mL	**BIOTIN**	**Serotonin Biotin** Yellow colored. Ready to use. Contains: < 0.1 % NaN_3.
1 x 0.2 mL	**ENZCONJ** **CONC**	**Enzyme Conjugate**, Concentrate (100x) Contains: streptavidin alkaline phosphatase, Tris buffer, HCl, < 0.1 % NaN_3.
1 x 7 x 0.5 mL	**CAL A-G**	**Standard A-G** 0; 0.08; 0.24; 0.73; 2.2; 6.6; 19.8 ng/mL 0; 0.45; 1.4; 4.1; 12.5; 37.4; 112.3 nmol/L Ready to use. Contains: Serotonin (acylated), phosphate buffer, < 0.1 % NaN_3.

CE

Revised 22 June 2012 rm (Vers. 11.1)

IVD

Quantity	Symbol	Component
1 x 2 x 0.5 mL	CONTROL 1+2 LYO	**Control 1+2**, lyophilized Contains: human serum, < 0.1 % NaN_3. Concentrations / acceptable ranges see QC Certificate.
1 x 3 mL	ACYLREAG	**Acylation Reagent** Acetic Acid Anhydride, acetone. Ready to use.
1 x 50 mL	ASSAYBUF CONC	**Assay Buffer** Concentrate (10x) Contains: phosphate buffer, BSA, < 0.1 % NaN_3.
1 x 50 mL	WASHBUF CONC	**Wash Buffer** Concentrate (20x) Contains: phosphate buffer, Tween, < 0.1 % Thimerosal
2 x 12 mL	PNPP SUBS	**PNPP Substrate Solution** Ready to use. Contains: p-nitrophenyl phosphate (PNPP).
1 x 15 mL	PNPP STOP	**PNPP Stop Solution** Ready to use. Contains: 1 M NaOH,
3 x	FOIL	**Adhesive Foil**

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (Multipette Eppendorf or similar devices, < 3% CV). Volume: 20; 25; 50; 100; 1000 µL
2. Disposable glass test tubes (12 x 75 mm)
3. Rack for test tubes
4. Orbital shaker (500 rpm)
5. Vortex mixer
6. Water bath, 37 °C
7. 8-Channel Micropipettor with reagent reservoirs
8. Wash bottle, automated or semi-automated microtiter plate washing system
9. Centrifuge; ≥ 1500 x g
10. Microtiter plate reader capable of reading absorbance at 405 nm (reference wavelength 600-650 nm)
11. Bidistilled or deionised water
12. Paper towels, pipette tips and timer

PROCEDURE NOTES

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-25 °C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each component and specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
4. Some components contain ≤ 250 µL solution. Take care that the solution is completely on the bottom of the vial before opening.
5. It is advised to determine samples in duplicate to be able to identify potential pipetting errors.
6. Use a pipetting scheme to verify an appropriate plate layout.
7. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.
8. Microplate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microplate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.
9. Humidity affects the coated wells/tubes. Do not open the pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch including the desiccant.
10. The relative centrifugal force (g) is not equivalent to rounds per minute (rpm) but it has to be calculated depending on the radius of the centrifuge.

PRE-TEST SETUP INSTRUCTIONS

- For manual and automatic version.

⚠ The contents of the kit for 96 determinations can be divided into 3 separate runs.

The volumes stated below are for one run with 4 strips (32 determinations).

If the customer wants to reduce the number of standards from 7 to 6 he can omit Standard G. The reportable range will then be reduced to 155 µg/L (plasma) or 706 µg/L (serum, urine, tissue homogenates, cell cultures supernatants).

The test procedure can be performed in a short version with 3.5 h incubation for serum, urine, platelets, tissue homogenates and cell culture supernatants BUT NOT FOR PLASMA, or in an alternative version with overnight incubation for same samples AND PLASMA. Plasma must ALWAYS be incubated over night.

CE

Revised 22 June 2012 rm (Vers. 11.1) **IVD**

Preparation of lyophilized or concentrated components

Dilute/ dissolve	Component		Diluent	Rela-tion	Remarks	Storage	Stability
15 mL	ASSAYBUF CONC	ad 150 mL	bidist. water	1:10	A yellowish-brown color may occur without influence of test results.	2 °C - 8 °C	2 weeks
15 mL	WASHBUF CONC	ad 300 mL	bidist. water	1:20		2 °C - 8 °C	4 weeks
	CONTROL 1+2 LYO	with 0.50 mL	bidist. water		Let stand for 15 min. Mix without foaming.	≤ -20°C (Aliquots)	until Exp. date
60 µL	ENZCONJ CONC	with 6.0 mL	diluted Assay Buffer	1:101	Prepare freshly and use only once.	18-25°C	2 h

Dilution of Samples

Samples suspected to contain concentrations higher than the highest standard have to be diluted with Assay Buffer.

Acylation of Samples and Controls (not Standards)

The following procedure must be performed in two variants:

Sample A: Serum, Urine, platelet-extract, tissue homogenate and controls

Sample B: platelet-free plasma

⚠ **Do not acylate the standards. They are already acylated!**

The sample preparation leads to a 107fold dilution for serum, urine, platelets, tissue homogenates, cell culture supernatants and controls and to a 23.5fold dilution of plasma samples. This has to be considered for the calculation of results.

Sample A: Serum, Urine, platelet-extract, tissue homogenate and controls

1. Pipette **20 µL** of each **Control and sample A** into **glass test tubes**.
2. Pipette **100 µL** of diluted **Assay Buffer** to each tube. Vortex.
3. Pipette **25 µL** of **Acylation Reagent** into each tube. Vortex each tube immediately after pipetting.
4. Cover tubes. **Incubate 15 min** at **37°C** in a waterbath.
5. Pipette **2 mL** of diluted **Assay Buffer** into each tube. Vortex.
6. **Centrifuge** all tubes for **10 min** at 1500 x g.

⚠ Prepared samples have to be assayed immediately. The supernatant is stable for only 1 h at 18-25°C.

Sample B: platelet-free plasma

1. Pipette **50 µL** of each **sample B** into **glass test tubes**.
2. Pipette **100 µL** of diluted **Assay Buffer** to each tube. Vortex.

3. Pipette **25 µL** of **Acylation Reagent** into each tube. Vortex each tube immediately after pipetting.
4. Cover tubes. **Incubate 15 min** at **37°C** in a waterbath.
5. Pipette **1 mL** of diluted **Assay Buffer** into each tube. Vortex.
6. **Centrifuge** all tubes for **10 min** at 1500 x g.

⚠ Prepared samples have to be assayed immediately. The supernatant is stable for only 1 h at 18-25°C.

TEST PROCEDURE

Short version (Note: only for samples A, but not for plasma)

1. Pipette **50 µL** of each **Standard,** acylated **Control and** acylated **sample** into the respective wells of the Microtiter Plate.
2. Pipette **50 µL** of **Serotonin Biotin** into each well.
3. Pipette **50 µL** of **Serotonin Antiserum** into each well.
4. Cover plate with adhesive foil. **Incubate 90 min. at RT (18 - 25°C) on an orbital shaker (500 rpm).**
5. Remove adhesive foil. Discard incubation solution. Wash plate **3 x** with **250 µL** of diluted **Wash Buffer**. Remove excess solution by tapping the inverted plate on a paper towel.
6. Pipette **150 µL** of freshly prepared **Enzyme Conjugate** into each well.
7. Cover plate with adhesive foil. **Incubate 60 min** at **RT (18 - 25°C) on an orbital shaker (500 rpm).**
8. Remove adhesive foil. Discard incubation solution. Wash plate **3 x** with **250 µL** of diluted **Wash Buffer**. Remove excess solution by tapping the inverted plate on a paper towel.
9. For adding of Substrate and Stop Solution use, if available, an 8-channel Micropipettor. Pipetting should be carried out in the same time intervals for Substrate and Stop Solution. Use positive displacement and avoid formation of air bubbles.
10. Pipette **200 µL** of freshly prepared **PNPP Substrate Solution** into each well.
11. **Incubate 60 min** at **RT (18-25°C)** on an orbital shaker (500 rpm).
12. Stop the substrate reaction by adding **50 µL** of **PNPP Stop Solution** into each well. Briefly mix contents by gently shaking the plate.
13. **Measure** optical density with a photometer at **405 nm** (Reference-wavelength: 600-650 nm) within **60 min** after pipetting of the Stop Solution.

Alternative Version with overnight incubation (Samples A AND B)

First Day

1. Pipette **50 µL** of each **Standard,** acylated **Control and** acylated **sample** into the respective wells of the Microtiter Plate.
2. Pipette **50 µL** of **Serotonin Biotin** into each well.
3. Pipette **50 µL** of **Serotonin Antiserum** into each well.
4. Cover plate with adhesive foil. Shake plate carefully. **Incubate 16-20 h (overnight)** at **2 °C - 8 °C**.

Second Day

1. Remove adhesive foil. Discard incubation solution. Wash plate **3 x** with **250 µL** of diluted **Wash Buffer**. Remove excess solution by tapping the inverted plate on a paper towel.
2. Pipette **150 µL** of freshly prepared **Enzyme Conjugate** into each well.
3. Cover plate with adhesive foil. **Incubate 60 min** at **RT (18-25°C)** on an orbital shaker (500 rpm).
4. Remove adhesive foil. Discard incubation solution. Wash plate **3 x** with **250 µL** of diluted **Wash Buffer**. Remove excess solution by tapping the inverted plate on a paper towel.
5. For adding of Substrate and Stop Solution use, if available, an 8-channel Micropipettor. Pipetting should be carried out in the same time intervals for Substrate and Stop Solution. Use positive displacement and avoid formation of air bubbles.
6. Pipette **200 µL** of freshly prepared **PNPP Substrate Solution** into each well.
7. **Incubate 30 min** at **RT (18-25°C)** on an orbital shaker (500 rpm).
8. Stop the substrate reaction by adding **50 µL** of **PNPP Stop Solution** into each well. Briefly mix contents by gently shaking the plate.
9. **Measure** optical density with a photometer at **405 nm** (Reference-wavelength: 600-650 nm) within **60 min** after pipetting of the Stop Solution.

QUALITY CONTROL

The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable standards/laws. All kit controls must be found within the acceptable ranges as stated on the labels and the QC certificate. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls.

It is recommended to participate at appropriate quality assessment trials.

In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

CALCULATION OF RESULTS

The obtained OD of the standards (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline, 4 Parameter Logisitcs or Logit-Log.
For the calculation of the standard curve, apply each signal of the standards (one obvious outlier of duplicates might be omitted and the more plausible single value might be used).
The concentration of the samples can be read directly from the standard curve.
Due to the dilution of samples the values have to be multiplied by the corresponding factor to obtain the serotonin concentrations in **ng/mL**:

Serum, urine, platelets, tissue homogenates, cell culture supernatants, controls:	**x 107**
Platelet-free plasma:	**x 23.5**

Results of samples of higher predilution have to be multiplied with the dilution factor.

The assay can be declared valid if the following criteria are met:

50% OD/OD_{max} (ED 50): 0.60 - 1.00 ng/mL (mean 0.8 ng/mL).

Δ OD Standard A - Standard G: ≥ 0.80 OD.

Conversion:

Serotonin (ng/mL) x 5.67 = nmol/L

Samples showing concentrations above the highest standard have to be diluted as described in PRE-TEST SETUP INSTRUCTIONS and reassayed.

Calculation for platelets

The content of serotonin in platelets is referred to 10^9 platelets. Following is given an example:

Serotonin concentration: 100 ng/mL.

Number of platelets in the PRP: 300 000/µL equivalent to 60 000 000/200µL PRP and 200 µL of extraction volume. When using 20 µL for the test this is a platelet equivalent of 6 x 10^6 platelets.

The serotonin content is referred to 1 mL. Therefore the used platelets equivalent of 20 µL has to be multiplied by 50.

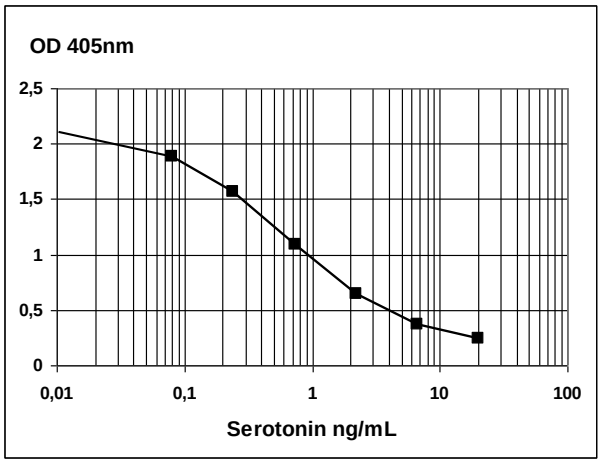
6 x 10^6 x 50 = 0.3 x 10^9 platelets/mL with a serotonin content of 100 ng.

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).

Typical Calibration Curve

(Example. Do not use for calculation!)

Standard	Serotonin (ng/mL)	OD_{Mean}	OD/OD_{max} (%)
A	0.0	2.118	100.0
B	0.08	1.883	88.9
C	0.24	1.568	74.0
D	0.73	1.089	51.5
E	2.2	0.641	30.3
F	6.6	0.369	17.4
G	19.8	0.245	11.6



EXPECTED VALUES

The results themselves should not be the only reason for any therapeutical consequences. They have to be correlated to other clinical observations and diagnostic tests.

Apparently healthy subjects show the following values: (97.5 % percentile)

Specimen	n	Unit	Mean	Range
Serum	99	ng/mL	88.6	30 – 200
Platelet-free Plasma	35	ng/mL	3.7	1.8 – 7.5
Platelets	35	ng/10^9 Platelets	490	217 – 861

It is recommended that each laboratory establishes its own range of normal values.

24 h Urine	49	µg/d	83.1	≤ 200

LIMITATIONS OF THE PROCEDURE

Specimen collection has a significant effect on the test results. See SPECIMEN COLLECTION AND STORAGE for details.
For cross reactivities, see PERFORMANCE.
Azide and thimerosal at concentrations > 0.1 % interfere in this assay and may lead to false results.
The following blood components do not have a significant effect (+/- 20 % of expected) on the test results up to the below stated concentrations:

Hemoglobin	8.33 mg/mL
Bilirubin	0.33 mg/mL

PERFORMANCE

Analytical Specificity (Cross Reactivity)	Substance	Cross Reactivity (%)		
	N-Acyl-Serotonin	100		
	5-HIAA	0.110		
	Melatonin	0.040		
	5-Methoxy-Tryptamine	0.015		
	3-Indolacrylic acid	< 0.01		
	Indole-3-pyruvic acid			
	3-Indolacetic acid			
	5-Metoxytryptophol			
	L-5-OH-Tryptophan			
Analytical Sensitivity (Limit of Detection)	Mean signal; (Zero-Standard) - 2SD (as read from the standard curve)			Overnight: 0.014 ng/mL
				Short version: 0.025 ng/mL
	Serum, Urine, Platelets, Tissue homogenates, Cell Culture Supernatants (multiplied by dilution factor)			Overnight: 1.50 ng/mL
				Short version: 2.68 ng/mL
	Plasma			Overnight: 0.33 ng/mL
Precision		Range (ng/mL)	CV (%)	
Intra-Assay	Serum	91 - 327	3.8 – 6.6	
	Urine	114 - 625	4.8 – 8.2	
	Plasma	7.1 - 247	3.7 – 11.5	
Inter-Assay	Serum	23 – 355	6.7 – 17.3	

	Urine	87 – 626	9.4 – 18.1	
	Plasma	8.9 - 30	6.8 – 17.9	
Linearity		Range (ng/mL)	Serial dilution up to	Range (%)
	Serum	226 - 1503	1:16	90 - 125
	Urine	677 - 1264	1:32	89 - 117
	Plasma	404 - 597	1:16	89 - 117
Recovery		Mean (%)	Range (%)	% Recovery after spiking
	Serum	104	85 - 119	
	Urine	98	85 - 116	
	Plasma	100	83 - 120	
Method Comparison versus HPLC / other ELISA	Serum	DRG-Assay = 0.90 x HPLC + 19.5		r = 0.945; n = 28
	Urine	DRG-Assay = 0.86 x ELISA + 20.0		r = 0.987; n = 32
	Platelets*	DRG-Assay = 0.992 x HPLC + 0.008		r = 0.992; n = 50
Method Comparison PNPP Substrate Solution RTU (Ready to use.) versus PNPP Substrate Tablets	Urine	DRG-Assay (pNPP RTU) = 0.97 x DRG-Assay (PNPP Substrate Tablets) + 4.8		r = 0.99; n = 32

*Reference: Kluge, H; Serotonin in Platelets; J Lab Med, 23 (6): 360-364 (1999)

SHORT PROTOCOL OVER NIGHT AND SHORT VERSION

Total assay time	**<5h (short version)**	**18-22 h (overnight)**	**18-22 h (overnight)**
Specimen	**Serum, Urine, Platlets-extract, Tissue homogenate and Controls**	**Serum, Urine, Platlets-extract, Tissue homogenate and Controls**	**Platlet-free plasma**
Sample pre-treatment	Do not acylate standards! They are already acylated.		
Acylation			
Sample volume	20 µL	20 µL	50 µL
Diluted Assay Buffer	100 µL	100 µL	100 µL
Acylation Reagent	25 µL	25 µL	25 µL
Incubation, waterbath	15 min 37°C	15 min 37°C	15 min 37°C

Diluted Assay Buffer	2000 µL	2000 µL	1000 µL
Centrifugation	10 min at 1500 x g	10 min at 1500 x g	10 min at 1500 x g

Microtiter Plate pipetting

Standards/ acylated sample	50 µL	50 µL	50 µL
Serotonin Biotin	50 µL	50 µL	50 µL
Serotonin Antiserum	50 µL	50 µL	50 µL

Sample incubation

Incubation time	90 min	16-20 h	16-20 h
Incubation temperature	RT (18-25°C)	2 °C - 8 °C	2 °C - 8 °C
Incubation condition	shaker 500 rpm	no shaker	no shaker
Washing steps	3 x 250 µL	3 x 250 µL	3 x 250 µL

Enzyme incubation

Diluted Enzyme Conjugate	150 µL	150 µL	150 µL
Incubation time	60 min	60 min	60 min
Incubation temperature	RT (18-25°C)	RT (18-25°C)	RT (18-25°C)
Incubation condition	shaker 500 rpm	shaker 500 rpm	shaker 500 rpm
Washing steps	3 x 250 µL	3 x 250 µL	3 x 250 µL

Substrat incubation

Pipetting volumen	200 µL	200 µL	200 µL
Incubation time	60 min	30 min	30 min
Incubation temperature	RT (18-25°C)	RT (18-25°C)	RT (18-25°C)
Incubation condition	shaker 500 rpm	shaker 500 rpm	shaker 500 rpm
Stop Solution	50 µL	50 µL	50 µL
Measure optical density	405 nm (reference wavelength: 600 – 650 nm)		

CE

Revised 22 June 2012 rm (Vers. 11.1) **IVD**

References

1. Lahiri, D. K., Ge, Y.-W., Sharman, E. H., Bondy, St. C., Age-related changes in serum melatonin in mice: higher levels of combined melatonin and 6-hydroxymelatonin sulfate in the cerebral cortex than serum, heart, liver and kidney tissues. J. Pineal Res. May 2004, Vol. 36, issue 4, 217-223
2. Bethea, C. L., Lu, N. Z., Reddy, A., Shlaes, T., Streicher, J. M., Whittemore, S. R., Characterization of reproductive steroid receptors and response to estrogen in a rat serotonergic cell line. Journal of Neuroscience Methods 127, 31-41 (2003). Address: Bethea, C. L., Oregon National Primate Research Center, Beaverton, USA.
3. Pan, J et al. Cystic Fibrosis Transmembrane Conductance regulator Modulates Neurosecretory Function in Pulmonary Neuroendocrine Cell-Related Tumor Cell Line Models. Am. J. Respir. Cell Mol. Biol. Vol. 27, 553 – 560 (2002)
4. Khan, I., Thomas, P., Disruption of Neuroendocrine Control of Luteinizing Hormone Secretion by Aroclor 1254 involves Inhibition of Hypothalamic Tryptophan Hydroxylase Activity. Biology of Reproduction, 64, 955-964 (2001). Address: Khan, I.A., University of Texas at Austin, U.S.A.
5. Harenberg, J, Huhle, G., Giese Ch., Wang, L., Feuring, M., Song, X., Hofmann, U.: Determination of serotonin release from platelets by enzyme immunoassay in the diagnosis of heparin-induced thrombocytopenia. British Journal of Hematology, 109, 182-186 (2000) Address: Harenberg J., University of Heidelberg, Germany
6. Kluge, H., Bolle, M., Reuter, R., Werner, S., Zahlten, W., Prudlo, J., Serotonin in Platelets: Comparative Analyses using New Enzyme Immunoassay and HPLC Test Kits and the Traditional Fluorimetric Procedure. J Lab Med, 23 (6): 360-364 (1999)
   Address: Kluge, H., University of Jena, Germany
7. Balaskas, E., Bamihas, G., Karamouzis M., Voyiatzis, G., Tourkantonis, A. Histamine and Serotonin in uremic pruritus: effect of ondansetron in CAPD-pruritic patients. Nephron, 78:395-402 (1998)
   Address: Elias Balaskas, MD Ahepa University Hospital, Thessaloniki, Greece
8. Sprott,H., Kluge,H., Franke,S, Hein,G. Altered Serotonin-Levels in Patients with Fibromyalgia. In: Journal of Musculoskeletal Pain, Abstracts from the 3rd World Congress on Myofascial Pain and Fibromyalgia San Antonio, Texas, USA, Editor: I. J. Russel, p. 65. (1995)
   Address: Kluge,H., University of Jena, Jena, Germany
9. Thomas Stratz, Wlodzimierz Samborski, Pawel Hrycaj, Thomas Pap, Stefan Mackiewicz, Pierre Mennet, Wolfgang Müller Die Serotoninkonzentration im Serum bei Patienten mit generalisierter Tendomyopathie (Fibromyalgie) und chronischer Polyarthritis. In: Medizinische Klinik, 88, 458-462 (1993).
   Address: Thomas Stratz, Hochrhein-Institut für Rheumaforschung und Rheumaprävention, Bad Säckingen/Rheinfelden, Germany

Rev. 6/20/12cc