DRG

DRG® 5-HIAA (EIA-1886)

CE

Revised 29 Oct. 2010 rm (Vers. 4.1)

IVD

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

1 INTENDED USE

Enzyme immunoassay for the *in-vitro-diagnostic* quantitative determination of 5-HIAA in human urine.

2 SUMMARY AND EXPLANATION

The primary carcinoid tumor is usually derived from the enterochromaffin cells of the midgut and is located most frequently in the terminal ileum. Carcinoid tumours generally secrete various amounts of indoles. The carcinoid syndrome is generally characterized by an increased urinary excretion of 5-hydroxy-3-indole acetic acid (5-HIAA), the end product of serotonin (5-HT) metabolism.
Traditionally, 5-hydroxy-3-indole acetic acid is assayed by diazotization with nitrosonaphtol to form a purple colour. However, it is well documented that many other substances present in the urine interfere with this reaction to give false-positive results. Attempts were made to overcome this problem by a combination of ion exchange chromatography and fluorometry. These methods, however, lack sensitivity and are time consuming. Recently, high performance liquid chromatographic analyses of 5-HIAA with fluorometry in the ultraviolet region of the spectrum or electrochemical detection have been described. Both methods require solvent extraction because of the numerous interfering compounds present in urine. The 5-HIAA enzyme immunoassay is a new and simple method for the quantification of this important marker of carcinoid syndrome in small urine samples.

3 TEST PRINCIPLE

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 anti-biotin 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.

4 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.
9. Excess Methylation Reagent should be destroyed by addition of 1 mL 0.1 M HCl and should be handled as chemical waste as well as excess Dilution Reagent.

5 STORAGE AND STABILITY

The kit is shipped at ambient temperature and should be stored at 2-8°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 2–8°C.

6 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).

⚠ **5-HIAA is light sensitive. Keep dark during sampling.**

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:	2-8°C	≤ -20°C (Aliquots)	Keep away from heat or direct sun light.
Stability:	7 d	3 mon	Avoid repeated freeze-thaw cycles.

7 MATERIALS SUPPLIED

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

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Quantity	Symbol	Component
1 x 12x8	MTP	**Microtiter Plate** Break apart strips. Coated with anti-rabbit IgG (goat, polyclonal).
1 x 5 mL	ANTISERUM	**5-HIAA Antiserum** Blue colored. Ready to use. Contains: Antiserum (rabbit), phosphate buffer, stabilizers.
1 x 5 mL	BIOTIN	**5-HIAA Biotin** Ready to use. Blue colored. Contains: phosphate buffer, stabilizers.
1 x 0.2 mL	ENZCONJ CONC	**Enzyme Conjugate**, Concentrate (100x) Contains: Streptavidin conjugated to alkaline phosphatase, Tris buffer, stabilizers
1 x 7 x 0.5 mL	CAL A-G	**Standard A-G** 0; 0.4; 1.0; 2.7; 7.8; 19.5; 55.5 mg/L 0; 2.1; 5.25; 14.4; 39.4; 105; 288 µmol/L Ready to use. Contains: 5-HIAA (methylated), stabilizers.
1 x 2 x 0.5 mL	CONTROL 1+2 LYO	**Control 1+2**, lyophilized Contains: human urine (normal and pathological). Concentrations / acceptable ranges see vial labels.
1 x 2 mL	METHYLREAG	**Methylation Reagent** Yellow colored. Ready to use. Contains: dichloromethane.
1 x 1 mL	HCL	**HCl** Ready to use. 0.1 M HCl.
1 x 50 mL	ASSAYBUF CONC	**Assay Buffer**, Concentrate (10x) Contains: phosphate buffer, BSA, stabilizers.
1 x 4 mL	DILREAG	**Dilution Reagent** Ready to use. Contains: N,N-dimethylformamide.
1 x 50 mL	WASHBUF CONC	**Wash Buffer**, Concentrate (20x) Contains: phosphate buffer, Tween, stabilizers.
1 x 9	PNPP SUBS	**PNPP Substrate Tablets** In one foil packet. Contains: p-nitrophenyl phosphate (PNPP).
1 x 27 mL	PNPP BUF	**PNPP Substrate Buffer** Ready to use. Contains: diethanolamine, water.
1 x 15 mL	PNPP STOP	**PNPP Stop Solution** Ready to use. Contains: 1 M NaOH, 0.25 M EDTA.
3 x	FOIL	**Adhesive Foil**

8 MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (Multipette Eppendorf or similar devices, < 3% CV). Volumes: 20; 25; 50; 100; 1000 µL
2. Disposable glass test tubes (12 x 75 mm)
3. Vortex mixer
4. 8-Channel Micropipettor with reagent reservoirs
5. Wash bottle, automated or semi-automated microtiter plate washing system
6. Microtiter plate reader capable of reading absorbance at 405 nm (reference wavelength 600-650 nm)
7. Bidistilled or deionised water
8. Ventilated hood
9. Paper towels, pipette tips and timer

9 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. It is advised to determine samples in duplicate to be able to identify potential pipetting errors.
5. Use a pipetting scheme to verify an appropriate plate layout.
6. 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.
7. 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.
8. 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 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 3000 µg/L.

10.1 Preparation of lyophilized or concentrated components

Dilute/ dissolve	Component		Diluent	Rela-tion	Remarks	Storage	Stability
15 mL	**Assay Buffer**	ad 150 mL	bidist. water	1:10	Mix vigorously.	2-8°C	2 w
	Controls	with 0.5 mL	0.1 M HCl		Let stand for 15 min. Mix without foaming.	≤ -20°C (Aliquots)	8 w
15 mL	**Wash Buffer**	ad 300 mL	bidist. water	1:20	Warm up at 37°C to dissolve crystals, if necessary. Mix vigorously.	2-8°C	4 w
60 µL	**Enzyme Conjugate**	with 6.0 mL	Assay Buffer (diluted)	1:101	Prepare freshly and use only once. Mix without foaming.	18-25°C	30 min
3	**PNPP Substrate Tablets**	with 8 mL	PNPP Substrate Buffer		Prepare freshly and use only once. Mix without foaming.	18-25°C	10 min

10.2 Dilution of Samples

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

11 TEST PROCEDURE

11.1 First Day

11.1.1 Dilution and Methylation of Controls and Patient Samples (not Standards)

The sample preparation leads to a 255fold dilution. This has already been considered in the standard concentrations.

⚠ **Do not methylate the Standards. They are already methylated.**

1. **Pipette 20 µL** of each **Control and sample** into the respective glass tubes.
2. *After this step work under a ventilated hood!*
3. **Pipette 50 µL** of **Dilution Reagent** into each tube. Vortex.
4. **Pipette 25 µL** of **Methylation Reagent** into each tube. Vortex each tube immediately after pipetting.
   ***Note**: The yellow colour of the reaction mixture has to remain stable. Immediate disappearence of colour indicates an excess of acid in the sample. In this case add another 25 µL of Methylation Reagent!*
5. Cover tubes. **Incubate 20 min** at **RT (18-25°C)**.
6. **Pipette 5 mL** of diluted **Assay Buffer** into each tube. Cup tube with stopper and turn every tube e.g. manually (or by a mixer) at least 5 x upside and down to achieve complete mixing. Vortex.
7. *After this step the ventilated hood can be left.*
8. Withdraw **50 µL** aliquots of supernatant and perform the ELISA immediately. The supernatant is stable for 1 h at RT (18-25°C) only.

11.1.2 ELISA

1. **Pipette 50 µL** of each **Standard, methylated Control and methylated patient sample** into the respective wells of the microtiter plate.
2. **Pipette 50 µL** of **5-HIAA Biotin** into each well.
3. **Pipette 50 µL** of **5-HIAA Antiserum** into each well.
4. Cover plate with adhesive foil. Shake plate carefully. **Incubate over night (16-20 h)** at **2-8°C**.

11.2 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 new adhesive foil. **Incubate 120 min** at **RT (18-25°C)** on an orbital shaker (500 rpm).
4. *Approx. 10 min before end of incubation prepare PNPP Substrate Solution.*

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. 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.
7. **Pipette 200 µL** of freshly prepared **PNPP Substrate Solution** into each well.
8. **Incubate 60 min** at **RT (18-25°C)** on an orbital shaker (500 rpm).
9. Stop the substrate reaction by adding **50 µL** of **PNPP Stop Solution** into each well. Briefly mix contents by gently shaking the plate.
10. **Measure** optical density with a photometer at **405 nm** (Reference-wavelength: 600-650 nm) within **60 min** after pipetting of the Stop Solution.

12 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 vial labels. 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.

13 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 from the standard curve.

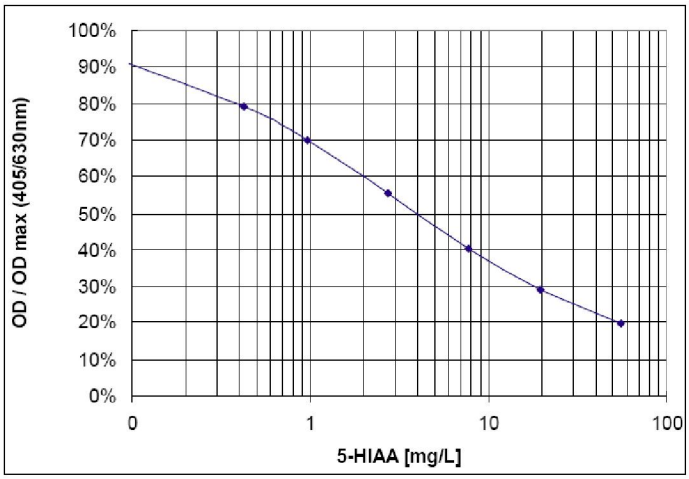
Samples showing concentrations above the highest standard have to be diluted as described in PRE-TEST SETUP INSTRUCTIONS and reassayed.

Calculate the 24 h excretion for each urine sample: µg/24h = µg/L x L/24h

Conversion: 5-HIAA (mg/L) x 5.25 = µmol/L

Typical Calibration Curve
(Example. Do not use for calculation!)

Standard	5-HIAA (mg/L)	Mean OD	OD/OD$_{max}$ (%)
A	0	1.906	100
B	0.4	1.511	79.3
C	1.0	1.331	69.8
D	2.75	1.054	55.3
E	7.5	0.767	40.3
F	20	0.553	29.0
G	55	0.377	19.8



14 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)

	Urine	
	mg/24h	µmol/d
5-HIAA	6 - 10	31.5 – 52.5

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

15 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.

16 PERFORMANCE

Analytical Specificity (Cross Reactivity)	Substance	Cross Reactivity (%)			
	5-HIAA	100			
	Serotonin-hydrochloride	9.5			
	Indole-3-Pyruvic acid	1.0			
	Melatonin	1.0			
	3-Indole-Acrylic acid	0.9			
	Tryptamine	0.8			
	3-Indole-Acetic acid	0.8			
	L-5-OH-Tryptophan	0.07			
	5-Methoxytryptophol	0.03			
	5-Methoxy-DL-Tryptophan	0.01			
	DL-Tryptophan	0.00			
Analytical Sensitivity (Limit of Detection)	0.06 mg/L	Mean signal (Zero-Standard) - 2SD			
Precision	Range (mg/L)	CV (%)			
Intra-Assay	5.4 – 36.7	4.8 – 7.1			
Inter-Assay	3.3 – 30.8	5.7 – 10.1			
Linearity	Range (mg/L)	Serial dilution up to	Range (%)		
	1.6 – 55.4	1:16	83 – 114		
Recovery	Mean (%)	Range (%)	% Recovery after spiking		
	95	81 - 118			
Method Comparison versus HPLC	DRG-Assay = 1.16 x HPLC - 1.58	r = 0.98; n = 61			

PRODUCT LITERATURE REFERNCES

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