EIA Serotonin

REF IM1749

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IMMUNOTECH s.r.o., Radiova 1122/1, 102 00 Prague 10, Czech Republic
www.beckmancoulter.com

EIA Serotonin

REF IM1749

ENZYME IMMUNOASSAY FOR THE IN VITRO DETERMINATION OF SEROTONIN IN BIOLOGICAL SAMPLES
For Research Use Only.

PRINCIPLE

The serotonin enzyme immunoassay (IM1749) is a Research Use Only product. Serotonin of samples or calibrators is chemically modified in acylated serotonin during a first step. Acylated serotonin is incubated in antibody coated wells in presence of alkaline phosphatase-acylated-serotonin conjugate. After incubation the wells are washed in order to remove non-bound components. The bound enzymatic activity is then measured after the addition of a chromogenic substrate.

WARNING AND PRECAUTIONS

General remarks:

- Do not mix the reagents from kits of different lots.
- The vials with calibrators should be opened as shortly as possible to avoid excessive evaporation.
- A standard curve must be established with each assay.
- It is recommended to perform the assay in duplicate.
- Bring all reagents to room temperature before pipeting.
- No eating, drinking, smoking or application of cosmetics should be carried out in the laboratory.
- No pipeting of any reagents or biological samples by mouth.
- Avoid direct contact with all caustic reagents by wearing gloves and laboratory garments.
- Decontaminate work surface with a 1:10 ratio of household bleach (sodium hypochlorite) and water.
- Waste should be discarded according to the country rules.

Sodium azide

Some reagents contain sodium azide as a preservative. Sodium azide can react with lead, copper or brass to form explosive metal azides. Sodium azide disposal must be in accordance with appropriate local regulations.

Materials of human origin

All blood samples should be handled as if capable of transmitting hepatitis or AIDS and waste should be discarded according to the country rules.

GHS HAZARD CLASSIFICATION

Conjugate	DANGER	
		
	H360	May damage fertility or the unborn child.
	P201	Obtain special instructions before use.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P308+P313	IF exposed or concerned: Get medical advice/attention.
		Boric Acid 0.5 - 1.0%
Calibrator	WARNING	
		
	H313	May be harmful in contact with skin
	H317	May cause an allergic skin reaction.
	H319	Causes serious eye irritation.
	H412	Harmful to aquatic life with long lasting effects.
	P273	Avoid release to the environment.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P337+P313	If eye irritation persists: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		2-Mercaptoethanol < 1%
		Citric Acid 1 - 5%
DMSO	WARNING	
		
	H227	Combustible Liquid
	H315	Causes skin irritation.
	H319	Causes serious eye irritation.
	H335	May cause respiratory irritation.
	P210	Keep away from heat, hot surfaces, and sparks. No smoking.
	P261	Avoid breathing vapours.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P304+P340	IF INHALED: Remove person to fresh air and keep at rest in a position comfortable for breathing.
	P312	Call a POISON CENTER or doctor/physician if you feel unwell.
	P337+P313	If eye irritation persists: Get medical advice/attention.
	P403+P233	Store in a well-ventilated place. Keep container tightly closed.
		Dimethyl Sulfoxide > 95%
Acylation Buffer	DANGER	
		
	H360	May damage fertility or the unborn child.
	P201	Obtain special instructions before use.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P308+P313	IF exposed or concerned: Get medical advice/attention.
		Boric Acid < 3%
Stop solution (NaOH)	DANGER	
		
	H314	Causes severe skin burns and eye damage.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P301+P330+P331	IF SWALLOWED: rinse mouth. Do NOT induce vomiting.
	P303+P361+P353	IF ON SKIN (or hair): Rinse skin with water.
	P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
	P310	Immediately call a POISON CENTER or doctor/physician.
		Sodium Hydroxide 1 - 5%
Wash Solution U (20X)	DANGER	

	H360	May damage fertility or the unborn child.
	P201	Obtain special instructions before use.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P308+P313	IF exposed or concerned: Get medical advice/attention.
		Boric Acid 0.1 - < 0.3%
		Sodium Borate Decahydrate 0.1 - < 0.3%
Substrate buffer	DANGER	

H316	Causes mild skin irritation.
H318	Causes serious eye damage.
H373	May cause damage to organs through prolonged or repeated exposure.
P280	Wear protective gloves, protective clothing and eye/face protection.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310	Immediately call a POISON CENTER or doctor/physician.
	Diethanolamine 5 - 10%

SDS Safety Data Sheet is available at beckmancoulter.com/techdocs

SPECIMEN COLLECTION, PROCESSING, STORAGE AND DILUTION

To avoid erroneously high levels of serotonin, a diet low in tryptophan is recommended for the 24 hours preceding sample collection. Molluscs, bananas, chocolats, pineapples, plums and dried fruits should not be eaten. It may be advisable under certain conditions to take samples consistently at the same time of day or, alternatively, to sample over a 24 hour period so as to exclude possible effects of a circadian rhythm on serotonin levels.

Obtain blood sample by venipuncture. Do not use a tourniquet.

An enzyme inhibitor solution may be used (per 2.5 mL of sample, 100 µL of 250 µM clorgyline, 250 µM chlorimipramine, 250 µM pargyline and 150 mM NaCl). It inhibits enzymatic oxidation and cellular uptake of serotonin. The inhibitor solution does not interfere with the assay.

Blood: Collect 2.5 mL of blood into a polystyrene tube containing cold lithium heparinate, mix gently by inversion. Blood samples, stored at 2-8°C, must be analyzed within 24 hours of collection. Dilute 1:100 in dilution buffer.

Serum: Collect 2.5 mL of blood into a glass tube and leave at room temperature for one hour. An inhibitor solution may be used. Centrifuge the sample at 1,700 g for 30 minutes at 2-8°C. Transfer the upper two-thirds of the serum to a polystyrene tube. Store at < -18°C until assay. Dilute 1:20 in dilution buffer.

Platelet-rich plasma: Collect 2.5 mL of blood into a cold (2-8°C) polystyrene tube containing sodium EDTA (5 mM), mix gently by inversion. Centrifuge immediately at 120 g, for 10 minutes at room temperature. Aspirate the upper three-quarters of the supernatant and store at < -18°C until assay. Dilute 1:100 in dilution buffer.

Platelet-poor plasma: Collect 2.5 mL of blood into a cold (2-8°C) polystyrene tube containing sodium EDTA (5 mM), mix gently by inversion. Immediately cool on ice. Within 20 minutes (platelets may lyse, if stored; hemolysed samples are unsuitable), centrifuge sample at 1,700 g for 30 minutes at 2-8°C. Transfer the upper two-thirds of the platelet-poor plasma to a polystyrene tube. Store at < -18°C until assay. Do not dilute.

Plasma: collect plasma using EDTA, citrate or heparin as anticoagulant. It is recommended to use EDTA plasma. Centrifuge for 15 minutes at 1 000 g within 1 hour of collection. Dilute 1:20 in dilution buffer.

Cerebrospinal fluid: Collect 1 mL of cerebrospinal fluid into a cold (2-8°C) polystyrene tube. Centrifuge at 1,500 g for 30 minutes at 2-8°C. Transfer the upper two-thirds of the fluid to a cold tube. Store at < -18°C until assay. Do not dilute.

Urine: Collect a 24 hour pooled urine specimen in a plastic container containing a bacteriostat such as boric acid or a few drops of toluene, etc. Determine total volume of 24 hour specimens. Mix well and take a 2 mL aliquot, store at 2-8°C and assay within one week (store at < -18°C, if sample is to be assayed later). Dilute 1:50 in dilution buffer.

Tissues: Collect the tissue into a cold polystyrene tube after weighing. Homogenize tissue in 0.2N $HClO_4$ (10 µL of 0.2N $HClO_4$ per mg of tissue) with sonicator. Centrifuge at 10,000 g for 5 min at 2-8°C. Collect and filter the supernatant through 0.22 µm filter. Neutralize (to pH 7-8) one volume of supernatant with one volume of 1 M borate buffer (pH 9.25). Centrifuge at 10,000 g for 1 min at 2-8°C. Store supernatant at < -18°C until assay.

Collection and storage: All reagents, i.e. calibrator, enzyme conjugate, dilution buffer and substrate, must be stored in the dark. To avoid oxidation of serotonin, protect samples at all stages of assay from bright light (e.g. by wrapping sample and dilution tubes in aluminium foil).

Avoid repeated freezing and thawing of calibrators and enzyme conjugate. Prepare aliquots if necessary.

MATERIALS PROVIDED

Before opening, all reagents of the kit are stable until the expiry date indicated on the kit labels, if stored at 2-8°C.

Reagents of the kit may be labelled with IVD symbol, this is due to manufacturer internal purposes only. Do not take the IVD symbol into account. This kit is for research use only - not for use in diagnostic procedures.

Expiry dates printed on vial labels apply to the long-term storage of components by the manufacturer only, prior to assembly of the kit. Do not take them into account.

Storage conditions for opened reagents are indicated below.

Storage conditions for reagents after reconstitution or dilution are indicated in paragraph Procedure.

Plate: 12 x 8 wells (ready-to-use)

Unused strips have to be stored at 2-8°C in the self-lock bag provided. After opening store strips at 2-8°C until the expiry date of the kit, for a maximum of 9 months.

Calibrator: one vial (lyophilized)

The vial contains bovine serum albumin.

Dilution buffer for calibrators and samples: one 25 mL vial (ready-to-use)

The vial contains bovine serum albumin and sodium azide. After opening store the dilution buffer at 2-8°C until the expiry date of the kit, for a maximum of 9 months.

Acylation reagent: one vial (powder)

Acylation buffer: one 5 mL vial (ready-to-use)

The vial contains bovine serum albumin in a borate buffer, pH 8.2. After opening store the acylation buffer at 2-8°C until expiration date of the kit, for a maximum of 9 months.

DMSO: one 3 mL vial (ready-to-use)

Serotonin-alkaline phosphatase conjugate: one vial (lyophilized)

The vial contains serotonin-alkaline phosphatase conjugate lyophilized in presence of bovine serum albumin.

Wash solution U (20X): one 50 mL vial

Concentrated solution has to be diluted before use.

Substrate buffer: one 30 mL vial (ready-to-use)

The substrate buffer is a diethanolamine-HCl solution. After opening store the substrate buffer at 2-8°C until expiration date of the kit, for a maximum of 9 months.

Substrate: two tablets

Stop solution: one 6 mL vial (ready-to-use)

This solution is a 1N NaOH solution. After opening store the stop solution at 2-8°C until expiration date of the kit, for a maximum of 9 months.

MATERIALS REQUIRED, BUT NOT PROVIDED

In addition to standard laboratory equipment, the following items are required:

- semi-automatic pipets (25, 50, 200, 300 µL)
- precision micropipets (20, 25, 100, 200 µL)
- orbital microplate shaker (350 rpm)
- vortex type mixer
- device for aspiration or microplate washer
- microtiter plate reader (405-414 nm)

PROCEDURE

Preliminary notes:

- Let the components of the kit equilibrate 30 minutes at room temperature before use.
- Dispensing of the reagents should not exceed 25 minutes. It must be done in the same order.
- Let DMSO solvent equilibrate at 18-25°C until completely melted.
- Avoid exposing the substrate to direct sunlight.

Preparation of reagents

- **Wash solution:** Pour the content of the vial into 950 mL of distilled water and homogenize. The diluted solution may be stored one month at 2-8°C or at < -18°C until expiration date of the kit, for a maximum of 9 months.
- **Conjugate:** Reconstitute the lyophilized conjugate with the volume of distilled water stated on the vial label. Wait 5-10 minutes before homogenizing. After reconstitution the conjugate is stable one week at 2-8°C or at < -18°C until the expiration date of the kit, for a maximum of 9 months.
- **Acylation reagent:** The content of the vial must be dissolved just before use in the volume of DMSO stated on the label. The reconstituted reagent is stable at < –18°C until the expiration date of the kit, for a maximum of 9 months. Avoid repeated freezing and thawing.
- **Substrate:** The substrate paranitrophenyl phosphate working solution is prepared at least 30 minutes before use, by dissolving one tablet in 15 mL of substrate buffer. The solution is stable 24 hours at 2–8°C or stored at < –18°C until the expiration date of the kit, for a maximum of 9 months.
- **Calibrators:** Reconstitute the lyophilized calibrator with the volume of distilled water stated on the vial label. Wait at least one-half hour after solubilization before dispensing. Mix gently to avoid foaming. Do not use a vortex system. Store at < –18°C in the dark, for a maximum of 9 months.
- From the 0.01 mM calibrator solution and the appropriate diluent, prepare a fresh dilution series in plastic tubes prior to each assay as indicated below. This dilution series cannot be stored.

Calibrator	Volume of serotonin solution	Dilution buffer
200 nM	40 µL of reconstituted calibrator (0.01 mM)	1960 µL
60 nM	300 µL of 200 nM calibrator	700 µL
18 nM	300 µL of 60 nM calibrator	700 µL
5.4 nM	300 µL of 18 nM calibrator	700 µL
1.6 nM	300 µL of 5.4 nM calibrator	700 µL
0 nM	—	1000 µL

Assay procedure

To avoid oxidation of serotonin, protect samples at all stages of assay from bright light (e.g. by wrapping samples and dilution tubes in aluminum foil).

Acylation	Immunological step	Enzymatic step
To clean plastic tubes, add	To coated wells, (except substrate blank wells) add:	To all wells add:
25 µL acylation buffer (except for tissue samples),	20 µL of acylated calibrator or sample	200 µL of substrate
100 µL of calibrator or sample,	200 µL of enzyme-conjugate	Incubate 1 hour in the dark with shaking at 18–25°C
25 µL of acylation solution and vortex immediately	Incubate for 3 hours in the dark at 18-25°C with shaking	Add
	Aspirate	50 µL of stop solution
	Rinse 3 times with 300 µL of wash solution	Read absorbance at 405-414 nm.

Tissues

Serotonin calibrator curve

Use as diluent for the calibrators a mixture of one volume of 0.2N $HClO_4$ and one volume of 1 M borate buffer (pH 9.25). Centrifuge at 10,000 g, 1 min at 2-8°C.

Sample preparation

For tissues, it is recommended that two assays per sample are run, one without dilution and one at a 1:5 dilution in the diluent.

RESULTS

Results are obtained from the standard curve by interpolation. The curve serves for the determination of analyte concentrations in samples measured at the same time as the calibrators.

Standard curve

Example of standard curve is given on the Certificate of Analysis provided with the kit and on the Beckman Coulter website (beckmancoulter.com/techdocs). The measured data are indicative only, do not use them for calculation of your results.

The results in the quality control department were calculated using *spline* curve fit with logit of B/B_0 on the vertical axis and log of analyte concentration of the calibrators on the horizontal axis.

Samples

Locate the mean absorbance on the vertical axis of the calibrator curve and read off the serotonin concentration on the horizontal axis. That value corresponds to nanomoles per liter (nM) of the sample.

Correction must be made for dilution of the samples as follows:

- Blood: result (nM) x 100
- Serum, Plasma: result (nM) x 20
- Platelet-rich plasma: result (nM) x 100
- Platelet-poor plasma: result (nM)
- Cerebrospinal fluid: result (nM)
- Urine: result (nM) x 50 x total volume (in liters) of 24 hour specimen = serotonin in nmoles per 24 hour.
- Tissue: result (nM) x 2 x 10^{-2} x 176.2 x dilution factor = serotonin in ng/g of tissue.

Concentrations are obtained as nM and may be converted to ng/mL by multiplying values by 0.176.

QUALITY CONTROL

Good laboratory practices imply that control samples be used regularly to ensure the quality of the results obtained. These samples must be processed exactly the same way as the assay samples, and it is recommended to analyze their results using appropriate statistical methods.

In case of packaging deterioration or if data obtained show some performance alteration, please contact your local distributor or use the following e-mail address: imunochem@beckman.com

PERFORMANCE CHARACTERISTICS

(For more details, see the data sheet "APPENDIX")

Representative data are provided for illustration only. Performance obtained in individual laboratories may vary.

Sensitivity

Analytical sensitivity: 0.95 nM

Specificity

The specificity of the EIA Serotonin was investigated with 8 compounds structurally related to acylated serotonin. The table in "Appendix" shows the ratio: Analog concentration/ Acylated serotonin concentration at 50% of maximal absorbance.

Precision

Intra-assay

Serum samples were assayed 25 times in the same series. The coefficients of variation were found below or equal to 19.54%.

Inter-assay

Serum samples were assayed in duplicate in 10 different series. Coefficients of variation were found below or equal to 19.72%.

Measurement range (from analytical sensitivity to the highest calibrator):

0.95 to 200 nM.

LIMITATIONS

The non-respect of the instructions in this package insert may affect results significantly.

Deterioration of the serotonin enzyme immunoassay is indicated if any of the liquid components become brownish, turbid or if a precipitate appears.

Interference

Do not use hemolyzed, lipemic or icteric samples.

APPENDIX

PERFORMANCE CHARACTERISTICS

Representative data are provided for illustration only. Performance obtained in individual laboratories may vary.

Specificity

Data on cross-reactivity with several related molecules are presented in the following table.

Analog	Cross-reactivity ratio*
Acylated serotonin	1.0
N-Succinyl serotonin	2.8
Acylated tryptamine	1,000
Acylated 5-methoxytryptamine	3,400
Acylated 5-hydroxytryptophan	6,700
Acylated 5-hydroxyindoleacetic acid	150,000
Acylated melatonin	172,000
Serotonin	230,000
Acylated tryptophan	1,000,000

· * Analog concentration (nmol/L) at 50% of B/B_0 divided by acylated serotonin concentration (nmol/L) at 50% of B/B_0.

Precision

Intra-assay

Serum	S1	S2	S3
Number of determinations	25	25	25
Mean value (nM)	6.69	18.27	83.49
C.V., (%)	19.54	4.82	4.48

Inter-assay

Serum	S1	S2	S3
Number of determinations	10	10	10
Mean value (nM)	4.36	15.71	56.19
C.V., (%)	19.72	8.98	4.13

Symbols Key

REF	Product Reference
CONTENTS	Contents
	Manufactured by
	Contains sufficient for <n> tests
SDS	Safety Data Sheets
	Consult Instructions for Use
	Temperature Range(s)
	Caution
	Expiration Date
LOT	Lot Number
	Date of Manufacture
	Biohazard
DANGER	Danger
CONJ	Conjugate
PLATE	Plate
IFU	Instruction for Use

REAG ACYL	Acylation Reagent
BUF ACYL	Acylation Buffer
BUF SUBS	Substrate Buffer
DMSO	Dimethyl Sulfoxide
Diluent	Diluent
SOLN WASH 20x	Wash Solution (20x)
SOLN STOP	Stopping Solution
CAL	Calibrator
SUBSTRATE	Substrate

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IMMUNOTECH s.r.o., Radiova 1122/1, 102 00
Prague 10, Czech Republic

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