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ab133053
Serotonin ELISA Kit

For quantitative detection of Serotonin in Serum, Platelets, Plasma and Urine.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

Abcam's Serotonin *in vitro* competitive ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of Serotonin in Serum, Platelets, Plasma and Urine.

A goat anti-rabbit IgG antibody has been precoated onto 96-well plates. Standards or test samples are added to the wells, along with an alkaline phosphatase (AP) conjugated-serotonin antigen and a polyclonal rabbit antibody specific to Serotonin. After incubation the excess reagents are washed away. pNpp substrate is added and after a short incubation the enzyme reaction is stopped and the yellow color generated is read at 405 nm. The intensity of the yellow coloration is inversely proportional to the amount of Serotonin captured in the plate.

Serotonin (5-hydroxytryptamine, 5-HT) is a monoamine found in the central nervous system, gastrointestinal tract, and blood with broad physiological functions as a neurotransmitter, in gastric motility, hemostasis, and cardiovascular integrity. Defects in serotonin signaling have been linked to a large number of complex behavioral disorders in humans, including anxiety and depression, autism, and eating disorders, and to neurodegenerative disorders such as Parkinson's. Platelets serve as the major reservoir of serotonin in the bloodstream. When activated, platelets release serotonin in to the bloodstream where it acts as a powerful vasoconstrictor. Treatment with Selective Serotonin Uptake Inhibitors (SSRIs) dramatically reduces platelet serotonin concentrations, and altered levels of serotonin in the circulatory system are implicated in such diverse conditions such as asthma, liver cirrhosis and carcinoid tumors.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed

Add standards and samples to appropriate wells.

Add prepared labeled AP-conjugate to appropriate wells.

Add Serotonin antibody to appropriate wells. Incubate at room temperature.

Add pNpp substrate to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C. Avoid multiple freeze-thaw cycles. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
Goat anti-rabbit IgG Microplate (12 x 8 wells)	96 wells	-20°C
Serotonin Alkaline Phosphatase Conjugate	2 x 150 ng	-20°C
Serotonin Antibody	2 vials	-20°C
Serotonin Standard	2 x 250 ng	-20°C
Assay Buffer	27 mL	-20°C
20X Wash Buffer Concentrate	27 mL	-20°C
pNpp Substrate	20 mL	-20°C
Stop Solution	5 mL	-20°C
Plate Sealer	2 x 1 unit	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Standard microplate reader - capable of reading at 405 nm, preferably with correction between 570 and 590 nm.
- Automated plate washer (optional).
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended when large sample sets are being analyzed.
- Eppendorf tubes.
- Microplate Shaker.
- Absorbent paper for blotting.
- Deionized water.

8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Standards can be made up in either glass or plastic tubes.
- Pre-rinse the pipette tip with the reagent, use fresh pipette tips for each sample, standard and reagent.
- Pipette standards and samples to the bottom of the wells and add the reagents to the side of the well to avoid contamination. If unsure, always change pipette tips between wells.
- This kit uses break-apart microtiter strips, which allow the user to measure as many samples as desired. Unused wells must be kept desiccated at 4°C in the sealed bag provided. The wells should be used in the frame provided.
- Care must be taken to minimize contamination by endogenous alkaline phosphatase. Contaminating alkaline phosphatase activity, especially in the substrate solution, may lead to high blanks. Care should be taken not to touch pipet tips and other items that are used in the assay with bare hands.
- Prior to addition of substrate, ensure that there is no residual wash buffer in the wells. Any remaining wash buffer may cause variation in assay results.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 Serotonin Antibody

Reconstitute one vial of Serotonin antibody with 3 mL of the assay buffer and vortex thoroughly. Unused reconstituted antibody should be discarded.

9.2 Serotonin Conjugate

Allow the Serotonin Alkaline Phosphatase Conjugate to equilibrate to room temperature. Reconstitute one vial of serotonin conjugate with 3 mL of the assay buffer. Mix thoroughly. Any unused conjugate should be aliquoted and re-frozen at or below -20°C.

9.3 1X Wash Buffer

Prepare the 1X Wash Buffer by diluting 5 mL of the 20X Wash Buffer Concentrate in 95 mL of deionized water. Mix thoroughly and gently.

9.4 Conjugate 1:20 Dilution for Total Activity Measurement

Prepare the Conjugate 1:20 Dilution by diluting 5 µL of the reconstituted conjugate with 95 µL of the assay buffer. The dilution should be used within three hours of preparation. This 1:20 dilution is intended for use in the Total Activity (TA) wells only.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Prepare serially diluted standards immediately prior to use.
- Diluted standards should be used within 60 minutes of preparation.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 Reconstitute one vial of Serotonin standard with 500 µL Assay Buffer. Vortex to ensure pellet is fully dissolved. This solution is **Standard 1**.

10.2 Label 5 tubes #2 – #6.

10.3 Add 375 µL of Assay Buffer into all tubes.

10.4 Prepare a 125 ng/mL **Standard 2** by adding 125 µL of the 500 ng/mL **Standard 1** to tube #2. Mix thoroughly and gently.

10.5 Prepare **Standard 3** by transferring 125 µL from Standard 2 to tube #3. Mix thoroughly and gently.

10.6 Using the table below as a guide, repeat for tubes #4 through# 6.

Standard #	Volume to dilute (µL)	Volume Diluent (µL)	Starting Conc. (ng/mL)	Final Conc. (ng/mL)
1	Step 10.1			500
2	125 µL Standard #1	375	500	125
3	125 µL Standard #2	375	125	31.25
4	125 µL Standard #3	375	31.25	7.81
5	125 µL Standard #4	375	7.81	1.95
6	125 µL Standard #5	375	1.95	0.49

11. Sample Preparation

The Serotonin ELISA kit is compatible with natural and recombinant Serotonin samples in serum, platelets, plasma and urine.

A minimum 1:16 dilution is required for serum, plasma, and urine samples. A minimum 1:2 is required for platelets. These are the minimum recommended dilution to remove matrix interference in the assay. Due to differences in samples, users must determine the optimal sample dilution for their experiments.

11.1 Platelets:

Platelets are isolated from whole blood and should be counted, prior to extracting the serotonin.

11.1.1 Blood should be collected by venipuncture into plastic tubes containing anticoagulant; either citrate or EDTA may be used.

11.1.2 Centrifuge at 1,600 x g for 15 minutes at room temperature.

11.1.3 Collect the white buffy coat layer with a plastic pipette and transfer to a fresh plastic tube note the volume collected.

11.1.4 Count platelets in a hemocytometer.

11.1.5 Wash the platelets with twice the original volume using physiological saline and centrifugation at 2,000 x g for 10 minutes. Repeat wash for a total of two washes.

11.1.6 Resuspend the platelet pellet back to the original volume using distilled water. This suspension is then frozen followed by thawing shortly before assay. The frozen suspension may be aliquoted and stored for up to two weeks.

11.2 Serum:

11.2.1 Collect whole blood in appropriate serum tubes.

11.2.2 Centrifuge at 1,000 x g for 15 minutes at room temperature.

11.2.3 Remove serum to a clean plastic tube.

11.2.4 Sample should be aliquoted and frozen within two hours of collection.

11.2.5 Samples may be stored frozen for up to two weeks.

11.3 Plasma:

11.3.1 Collect whole blood in appropriate tubes.

11.3.2 Centrifuge at 1,000 x g for 15 minutes at room temperature.

11.3.3 Remove plasma to a clean plastic tube.

11.3.4 Sample should be aliquoted and frozen within two hours of collection.
11.3.5 Samples may be stored frozen for up to two weeks.

11.4 Urine:

11.4.1 Collect spontaneous or 24 hour urine in a bottle.
11.4.2 Centrifuge at 1,000 x g for 10 minutes at room temperature.
11.4.3 Remove supernatant to a clean plastic tube.
11.4.4 Sample should be aliquoted and frozen and stored for up to two weeks.

12. Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents
- Unused well strips should be returned to the plate packet and stored at 4°C
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay.

Recommended plate layout

	1	2	3	4
A	B_s	Std 1	Std 5	Sample 3
B	B_s	Std 1	Std 5	Sample 3
C	TA	Std 2	Std 6	etc
D	TA	Std 2	Std 6	etc
E	NSB	Std 3	Sample 1	
F	NSB	Std 3	Sample 1	
G	B_0	Std 4	Sample 2	
H	B_0	Std 4	Sample 2	

Key:

B_s = Blank; contains substrate only.

TA = Total Activity; contains conjugate (5 µL) and substrate.

NSB = Non-specific binding; contains assay buffer, conjugate and substrate.

B_0 = 0 pg/mL standard; contains assay buffer, conjugate, antibody and substrate.

13. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Refer to the recommended plate layout in Section 12 before proceeding with the assay

13.1 Add 150 µL of the Assay Buffer into the NSB (non-specific binding) wells.

13.2 Add 100 µL of the Assay Buffer into the B_0 (0 pg/mL standard) wells.

13.3 Add 100 µL of prepared standards and 100 µL diluted samples to the appropriate wells.

13.4 Add 50 µL of Serotonin Alkaline Phosphatase Conjugate into NSB, B_0, standard and sample wells, i.e. not TA (Total Activity) and B_s (blank) wells.

13.5 Add 50 µL of Serotonin antibody into B_0, standard and sample wells, i.e. not B_s, TA and NSB wells.

13.6 Incubate the plate at room temperature on a plate shaker for 2 hours at ~500 rpm. The plate may be covered with the plate sealer provided.

13.7 Empty the contents of the wells and wash by adding 400 µL of 1X Wash Buffer to every well. Repeat the wash 2 more times for a total of 3 Washes. After the final wash, empty or aspirate the wells, and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.

13.8 Add 5 µL of the diluted (1:20) Serotonin Alkaline Phosphatase Conjugate to the TA well only.

13.9 Add 200 µL of the pNpp Substrate solution to every well. Incubate at room temperature for 1 hour without shaking.

13.10 Add 50 µL Stop Solution into each well. The plate should be read immediately.

13.11 After blanking the plate reader against the blank (B_s) wells, read optical density at 405 nm, preferably with correction between 570 and 590 nm. If the plate reader is not able to be blanked against the B_s wells, manually subtract the mean optical density of the blank wells from all readings.

The optimal speed for each shaker will vary and may range from 120 – 700 rpm. The speed must be set to ensure adequate mixing of the wells, but not so vigorously that the contents of the wells splash and contaminate other wells.

14. Calculations

A four parameter algorithm (4PL) provides the best fit, though other equations can be examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 4 parameter logistic). Interpolate protein concentrations for unknown samples from the standard curve plotted.

14.1 Calculate the average net absorbance measurement (Average Net OD) for each standard and sample by subtracting the average NSB absorbance measurement from the average absorbance measurement (Average OD) for each standard and sample.
Average Net OD = Average Bound OD - Average NSB OD

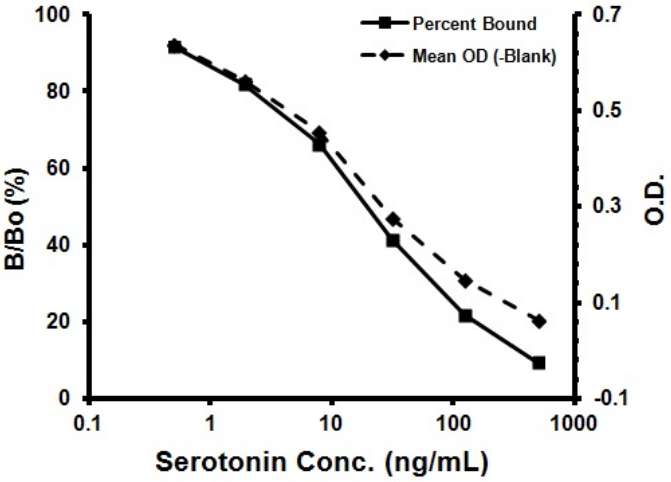
14.2 Calculate the binding of each pair of standard wells as a percentage of the maximum binding wells (B_0), using the following formula:
Percent Bound = (Net OD/ Net Bo OD) x 100

14.3 Plot the Percent Bound (B/B_0) versus concentration of serotonin for the standards. Approximate a straight line through the points. The concentration of serotonin in the unknowns can be determined by interpolation.

Samples producing signals greater than that of the highest standard should be further diluted and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.

15. Typical Data

Typical standard curve – data provided **for demonstration purposes only.** A new standard curve must be generated for each assay performed.



Sample	Mean OD (-Blank)	% Bound	Serotonin ng/mL
B_s	(0.088)	-	-
TA	0.997	-	-
NSB	0.008	0	-
Standard 1	0.063	9.4	500
Standard 2	0.147	21.8	125
Standard 3	0.277	41.3	31.3
Standard 4	0.455	66.2	7.8
Standard 5	0.562	81.8	1.9
Standard 6	0.637	91.6	0.5
B_0	0.691	100	0

Figure 1. Example of Serotonin standard curve.

16. Typical Sample Values

SENSITIVITY –

The sensitivity, defined as 2 standard deviations from the mean signal at zero, was determined from 7 independent standard curves. The standard deviation was determined from 14 zero standard replicates to be 0.293 ng/mL.

SAMPLE RECOVERY –

Sample Type	Spike Concentration (ng/mL)	Average % Recovery	Recommended Dilution
Human Platelets	100	106	1:16
	20	109	
	5	107	
Human Serum	100	95	1:16
	20	95	
	5	83	
Human Citrate Plasma	100	102	1:16
	20	101	
	5	83	
Human Urine	100	107	1:16
	20	91	
	5	104	

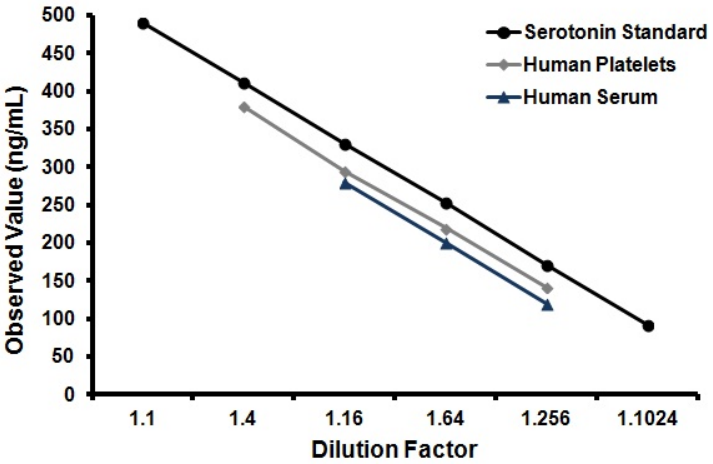
LINEARITY OF DILUTION –

Human samples containing serotonin were serially diluted 1:2 in the assay buffer and measured in the assay. Results are shown in the table below.

	Average % of Expected			
Dilution	Platelets	Serum	Plasma	Urine
Neat	88	-	-	-
1:2	103	-	-	-
1:4	115	-	-	-
1:8	114	93	-	88
1:16	117	118	115	107
1:32	112	116	110	105
1:64	100	106	73	103

PARALLELISM –

Dose-response curves from Human platelets and Human serum diluted into assay buffer were compared to the Serotonin standard curve. The parallel response indicates the standard effectively mimics the native protein.



PRECISION –

Intra-Assay

	Serotonin (ng/mL)	Intra-Assay %CV
Low	13.5	11.0
Medium	53.8	5.8
High	346.1	4.2

Inter-Assay

	Serotonin (ng/mL)	Inter-Assay %CV
Low	13.2	12.7
Medium	53.9	18.4
High	358.0	16.2

17. Assay Specificity

The Serotonin ELISA Kit recognizes natural and recombinant forms of Serotonin.

Compound	Cross Reactivity
N-Acetyl Serotonin	17%
5-Hydroxy-L-tryptophan	0.4%
Tryptamine	0.1%
5-Hydroxyindoleacetic acid	0.03%
Melatonin	0.01%
Tyramine	<0.004%
Tryptophan	<0.004%

Please contact our Technical Support team for more information.

18. Troubleshooting

Problem	Reason	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Samples give higher value than the highest standard	Starting sample concentration is too high.	Dilute the specimens and repeat the assay
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the kit	Store the all components as directed.

19. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)