



Product Manual

Serotonin ELISA kit

Catalog #: ADI-900-175

96 well enzyme-linked immunosorbent assay kit

For use with serum, platelets, plasma and urine

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Carefully note the handling and storage conditions of each kit component.



Please read entire booklet before proceeding with the assay.



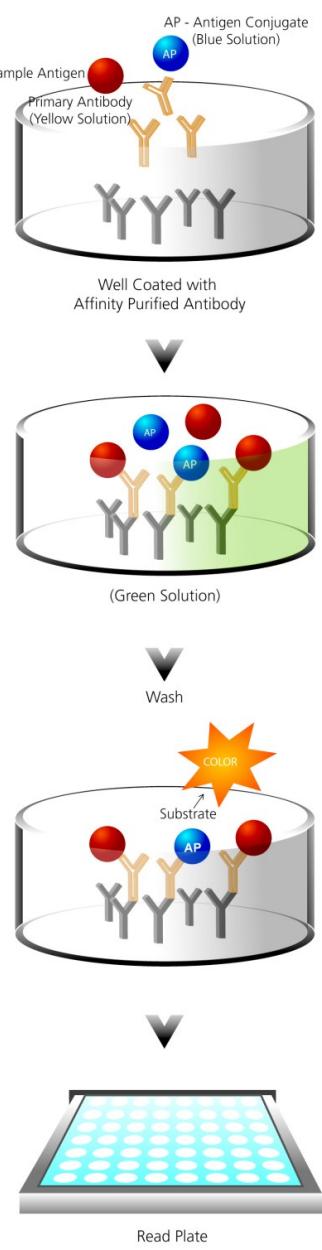
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INTRODUCTION

The Serotonin ELISA kit is a complete kit for the quantitative determination of serotonin in platelets, serum, plasma and urine. Please read the entire kit insert before performing this assay.

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.¹ Defects in serotonin signaling have been linked to a large number of complex behavioral disorders in humans, including anxiety and depression, autism, and eating disorders², and to neurodegenerative disorders such as Parkinson's³. Platelets serve as the major reservoir of serotonin in the bloodstream. When activated, platelets release serotonin into the bloodstream where it acts as a powerful vasoconstrictor⁴. Treatment with Selective Serotonin Uptake Inhibitors (SSRIs) dramatically reduces platelet serotonin concentrations⁵, and altered levels of serotonin in the circulatory system are implicated in such diverse conditions such as asthma⁶, liver cirrhosis⁷ and carcinoid tumors⁸.



PRINCIPLE OF THE ASSAY

1. Standards and samples are added to wells coated with a GxR IgG antibody. A solution of serotonin conjugated to alkaline phosphatase is then added, followed by a solution of rabbit polyclonal antibody to serotonin.
2. During a simultaneous incubation at room temperature the antibody binds, in a competitive manner, the serotonin in the sample or conjugate. The plate is washed, leaving only bound serotonin.
3. pNpp substrate solution is added. The substrate generates a yellow color when catalyzed by the alkaline phosphatase on the serotonin conjugate.
4. Stop solution is added. The yellow color is read at 405nm. The amount of signal is indirectly proportional to the amount of serotonin in the sample.

MATERIALS SUPPLIED

1. Assay Buffer

27 mL, Component Number 80-2192

Tris buffer containing proteins and preservative

2. Serotonin Standard

Component Number 80-2189

Two vials each containing 250ng lyophilized serotonin

3. Goat anti-Rabbit IgG Microtiter Plate

One plate of 96 wells, Component Number 80-0060

A clear plate of break-apart strips coated with a goat anti-rabbit polyclonal antibody

4. Serotonin Antibody

Component Number 80-2190

Two vials containing lyophilized serotonin polyclonal antibody

5. Serotonin Conjugate

Component Number 80-2191

Two vials each containing 150ng of lyophilized serotonin conjugated to alkaline phosphatase

6. Wash Buffer Concentrate

27 mL, Component Number 80-1286

Tris buffered saline containing detergents



Do not mix components from different kit lots or use reagents beyond the expiration date of the kit.



The standard should be handled with care due to the known and unknown effects of the antigen.



Avoid contamination by endogenous alkaline phosphatase. Do not expose reagents or supplies to bare skin.



Activity of conjugate is affected by concentrations of chelators > 10mM (such as EDTA and EGTA).



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- 7. pNpp Substrate**
20 mL, Component Number 80-0075
A solution of p-nitrophenyl phosphate
- 8. Stop Solution**
5 mL, Component Number 80-0247
A solution of trisodium phosphate in water
- 9. Serotonin Assay Layout Sheet**
1 each, Component Number 30-0283
- 10. Plate Sealer**
2 each, Component Number 30-0012



Stop solution is caustic. Keep tightly capped.



Storage temp

STORAGE

All components of this kit are shipped at -20°C.

Upon receipt the kit components can be stored at the temperature indicated on the component label or the whole kit can continue to be stored at -20°C until first use.

ADDITIONAL MATERIALS NEEDED

1. Deionized or distilled water
2. Precision pipets for volumes between 5 µL and 1,000 µL
3. Repeater pipet for dispensing 50 µL and 200 µL
4. Disposable beakers for diluting buffer concentrates
5. Graduated cylinders
6. Microplate shaker
7. Lint-free paper toweling for blotting
8. Microplate reader capable of reading at 405nm

SAMPLE HANDLING



If buffers other than those provided are used in the assay, the end-user must determine the appropriate dilution and assay validation.



Samples must be stored frozen at or below -20° to avoid loss of bioactive analyte. Repeated freeze/thaw cycles should be avoided.

The assay is suitable for the measurement of serotonin in platelets, serum, citrate plasma, and urine. Due to the conserved nature of serotonin between species, this kit is not species specific. However, samples containing rabbit IgG will interfere in the assay due to the GxR IgG coated plate. This assay is not recommended for use with hemolytic or lipemic samples or with Heparinized plasma. Prior to assay, frozen samples should be brought to 4°C and centrifuged, if necessary, to isolate residual debris.

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 particular experiments.

Protocol for Platelets

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

1. Blood should be collected by venipuncture into plastic tubes containing anticoagulant, either citrate or EDTA may be used.
2. Centrifuge at 1600 x g for 15 minutes at room temperature.
3. Collect the white buffy coat layer with a plastic pipette and transfer to a fresh plastic tube note the volume collected.
4. Count platelets in a hemocytometer.
5. Wash the platelets with twice the original volume using physiological saline and centrifugation at 2000 x g for 10 minutes. Repeat wash for a total of two washes.
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.

* The plasma layer may also be collected and aliquoted and stored frozen for up to two weeks.



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Protocol for Serum

1. Collect whole blood in appropriate serum tubes.
2. Centrifuge at 1000 x g for 15 minutes at room temperature.
3. Remove serum to a clean plastic tube.
4. Sample should be aliquoted and frozen within two hours of collection.
5. Samples may be stored frozen for up to two weeks.

Protocol for Plasma

1. Collect whole blood in appropriate tubes.
 2. Centrifuge at 1000 x g for 15 minutes at room temperature.
 3. Remove plasma to a clean plastic tube.
 4. Sample should be aliquoted and frozen within two hours of collection.
 5. Samples may be stored frozen for up to two weeks.
- * Plasma may also be collected as a part of the platelet protocol.

Protocol for Urine

1. Collect spontaneous or 24 hour urine in a bottle.
2. Centrifuge at 1000 x g for 10 minutes at room temperature.
3. Remove supernatant to a clean plastic tube.
4. Sample should be aliquoted and frozen and stored for up to two weeks.

REAGENT PREPARATION



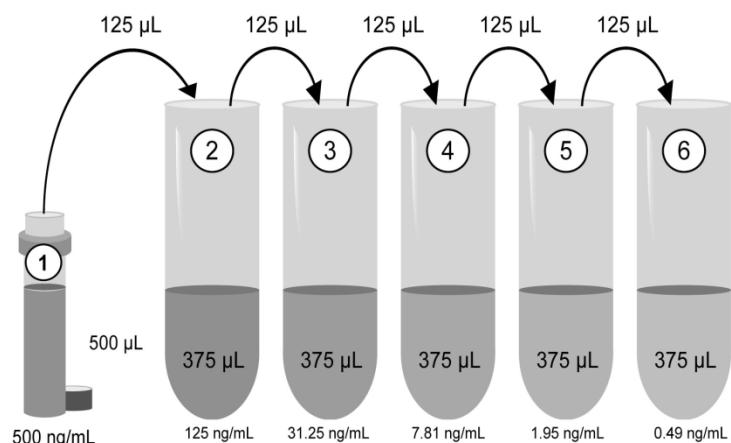
Glass or polypropylene tubes may be used for standard preparation. Avoid polystyrene.

1. Wash Buffer

Prepare the wash buffer by diluting 5 mL of the supplied Wash Buffer Concentrate with 95 mL of deionized water. This can be stored at room temperature until the kit expiration, or for 3 months, whichever is earlier.

2. Serotonin Standard

Reconstitute one vial of Serotonin standard with 500 μ L of the assay buffer. Vortex to ensure the entire cake is dissolved, this is standard #1. Label five 12 x 75 mm tubes #2 through #6. Pipet 375 μ L of the assay buffer into tubes #2 through #6. Remove 125 μ L from standard #1 and add to tube #2. Vortex thoroughly. Add 125 μ L from tube #2 to tube #3. Vortex thoroughly. Continue this for tubes #4 through #6.



Diluted standards should be used within 60 minutes of preparation. The concentrations of Serotonin in the tubes are labeled above.

3. Serotonin Antibody

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

4. Serotonin Conjugate

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

5. Conjugate 1:20 Dilution for Total Activity Measurement

Prepare the Conjugate 1:20 Dilution by diluting 5 μ L of the reconstituted conjugate with 95 μ L 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 wells ONLY.**

ASSAY PROCEDURE



Bring all reagents to room temperature for at least 30 minutes prior to opening.



Pre-rinse each pipet tip with reagent. Use fresh pipet tips for each sample, standard, and reagent.



All standards and samples should be run in duplicate.



Pipet the reagents to the sides of the wells to avoid possible contamination.



Prior to the addition of substrate, ensure there is no residual wash buffer in the wells. Remaining wash buffer may cause variation in assay results.

Refer to the Assay Layout Sheet to determine the number of wells to be used. Remove the wells not needed for the assay and return them, with the desiccant, to the mylar bag and seal. Store unused wells at 4°C.

1. Pipet 150 µL of the assay buffer into the NSB (non-specific binding) wells.
2. Pipet 100 µL of the assay buffer into the Bo (0ng/mL standard) wells.
3. Pipet 100 µL of Standards #1 through #6 to the bottom of the appropriate wells.
4. Pipet 100 µL of the samples to the bottom of the appropriate wells.
5. Pipet 50 µL of the conjugate into each well except the TA and Blank wells.
6. Pipet 50 µL of the antibody into each well except the Blank, TA, and NSB wells.
7. Seal the plate. Incubate for 2 hours on a plate shaker (~500rpm) at room temperature.
8. Empty the contents of the wells and wash by adding 400 µL of wash buffer to every well. Repeat 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.
9. Pipet 5 µL of the conjugate (diluted 1:20) to the TA wells.
10. Add 200 µL of the substrate solution into each well.
11. Incubate for 1 hour at room temperature with shaking.
12. Pipet 50 µL stop solution into each well.
13. After blanking the plate reader against the substrate blank, read optical density at 405nm. If plate reader is not capable of adjusting for the blank, manually subtract the mean OD of the substrate blank from all readings.

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

SAMPLE RECOVERIES

After diluting each individual sample to read within the dynamic range of the assay, recombinant serotonin was spiked at high, medium and low concentrations. Endogenous serotonin was subtracted from the spiked values and the average recovery in each of the spiked matrices was compared to the recovery of identical spikes in the assay buffer. The mean and the range percent recovery at the three concentrations are indicated below for each matrix.

Sample Matrix	Dilution	Spike Concentration	Recovery of Spike (Range)
Human Platelets (n=5)	1:16	100 ng/mL	106% (99-113%)
		20 ng/mL	109% (99-115%)
		5 ng/mL	107% (98-117%)
Human Serum (n=5)	1:16	100 ng/mL	95% (87-103%)
		20 ng/mL	95% (88-101%)
		5 ng/mL	83% (77-86%)
Human Citrate Plasma (n=5)	1:16	100 ng/mL	102% (93-113%)
		20 ng/mL	101% (94-117%)
		5 ng/mL	83% (62-114%)
Human Urine (n=3)	1:16	100 ng/mL	107% (97-120%)
		20 ng/mL	91% (80-106%)
		5 ng/mL	104% (66-157%)

CALCULATION OF RESULTS



Make sure to multiply sample concentrations by the dilution factor used during sample preparation.

Several options are available for the calculation of the concentration of Serotonin in the samples. We recommend that the data be handled by an immunoassay software package utilizing a 4 parameter logistic curve fitting program. If data reduction software is not readily available, the concentration of Serotonin can be calculated as follows:

1. Calculate the average net OD for each standard and sample by subtracting the average NSB OD from the average OD for each standard and sample.

$$\text{Average Net OD} = \text{Average OD} - \text{Average NSB OD}$$

2. Calculate the binding of each pair of standard wells as a percentage of the maximum binding wells (Bo), using the following formula:

$$\text{Percent Bound} = \frac{\text{Net OD}}{\text{Net Bo OD}} \times 100$$

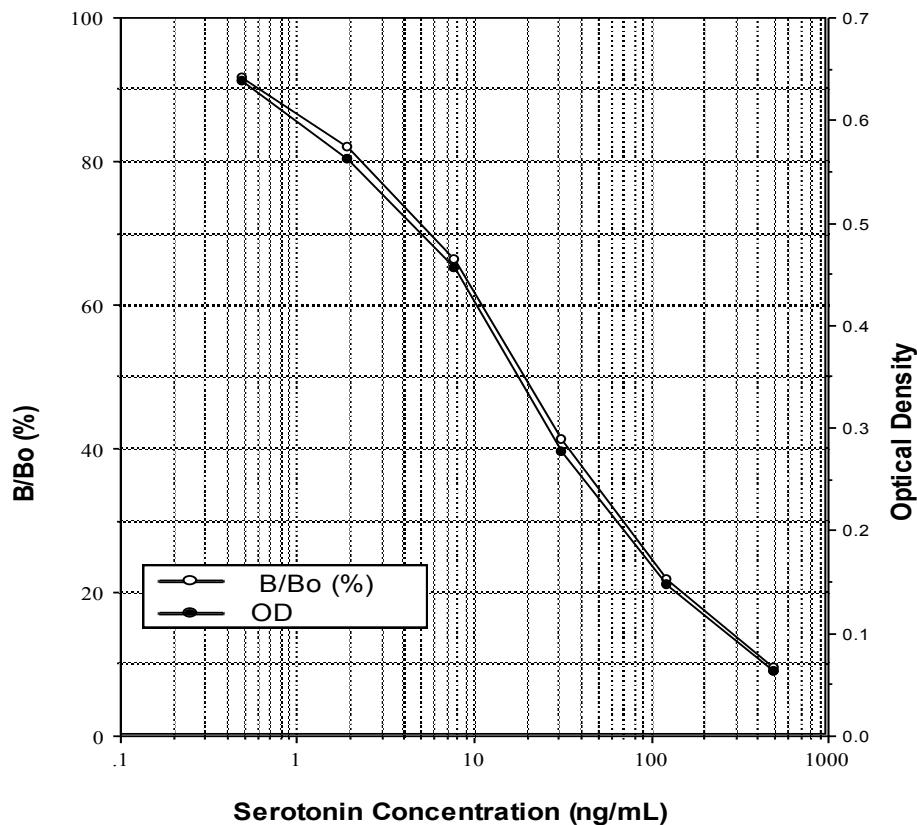
3. Plot the Percent Bound (B/Bo) versus concentration of Serotonin for the standards. Approximate a straight line through the points. The concentration of Serotonin of the unknowns can be determined by interpolation.

Samples with concentrations outside of the standard curve range will need to be re-analyzed using a different dilution.

TYPICAL RESULTS

The results shown below are for illustration only and should not be used to calculate results from another assay.

Sample	Average NetOD	Percent Bound	Serotonin (ng/mL)
Blank (mean)	(0.088)	---	---
TA	0.997	---	---
NSB	0.008	0%	---
Bo	0.691	100%	0
S1	0.063	9.4%	500
S2	0.147	21.8%	125
S3	0.277	41.3%	31.3
S4	0.455	66.2%	7.8
S5	0.562	81.8%	1.9
S6	0.637	91.6%	0.5





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PERFORMANCE CHARACTERISTICS

Specificity

The cross reactivities for a number of related compounds were determined by diluting the cross reactants in the kit assay buffer at a concentration of one hundred times the high standard. These samples were then measured in the assay.

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%

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. The sensitivity of the assay was determined to be 0.293 ng/mL.

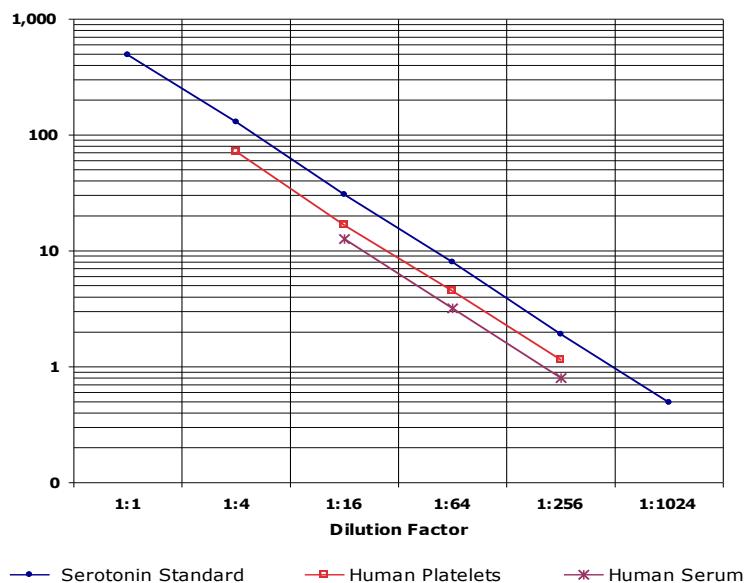
Dilutional Linearity

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

Dilution	Average % of Expected			
	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 precision was determined by assaying 20 replicates of three buffer controls containing serotonin in a single assay.

ng/mL	%CV
346.1	4.2
53.8	5.8
13.5	11.0

Inter-assay precision was determined by measuring buffer controls of varying serotonin concentrations in multiple assays over several days.

ng/mL	%CV
358.0	16.2
53.9	18.4
13.2	12.7



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