Product Manual

Revision date: 09/Oct/2018

5-Hydroxyindoleacetic Acid (5-HIAA) CLIA Kit

Catalog No.: abx490349

Size: 96T

Range: 1.56 pg/ml - 400 pg/ml

Sensitivity: < 0.59 pg/ml

Storage: Store standard, detection reagent A, detection reagent B and the 96-well plate at -20°C, and the rest of the kit components at 4°C.

Application: For quantitative detection of 5-HIAA in Serum, Plasma, Urine and other biological fluids.

Introduction: 5-Hydroxyindoleacetic acid (5-HIAA) is the main metabolite of serotonin. In chemical analysis of urine samples, 5-HIAA is used to determine serotonin levels in the body. 5-HIAA is tested by 24-hour urine samples combined with an acidic additive to maintain pH below 3. Certain foods and drugs are known to interfere with the measurement. 5-HIAA levels can vary depending on other complications, including tumors, renal malfunction, and small bowel resection. Since 5-HIAA is a metabolite of serotonin, testing is most frequently performed for the diagnosis of carcinoid tumors of the enterochromaffin (Kultschitzsky) cells of the small intestine, which release large amounts of serotonin. Values greater than 25 mg per 24 hours (higher if the patient has malabsorption) are strong evidence for carcinoid. The normal range is 2 to 6 mg per 24 hours. Low levels of 5-HIAA in the cerebrospinal fluid have been associated with aggressive behavior and suicide by violent means, correlating with diminished serotonin levels. Elevated serotonin (hyperserotonemia) is one of the most common biological findings in autism and 5-HIAA may be elevated in patients with autistic spectrum disorders

Principle of the Assay

This kit is based on competitive chemiluminescent immunoassay technology. 5-HIAA antibody is pre-coated onto a 96-well plate. A competitive inhibition reaction is launched between biotin labeled 5-HIAA and unlabeled 5-HIAA with the pre-coated antibody specific to 5-HIAA. After washing away the unbound conjugates, avidin conjugated to Horseradish Peroxidase is added to each microplate well and incubated. After the mixture of substrate A and B is added only wells that contain biotin labeled 5-HIAA will produce chemiluminescence. The intensity of the emitted light is inversely proportional to the amount of 5-HIAA in the sample or standard.

Kit components

1. One pre-coated 96-well microplate (12 × 8 well strips)
2. Standard: 2 tubes
3. Standard Diluent Buffer: 20 ml
4. Wash Buffer (30X): 20 ml. Dilution: 1:30
5. Detection Reagent A (100X): 120 µl
6. Detection Reagent B (100X): 120 µl
7. Diluent A: 12 ml
8. Diluent B: 12 ml
9. Substrate A: 10 ml
10. Substrate B: 2 ml
11. Plate sealer: 4

Material Required But Not Provided

1. 37°C incubator
2. Luminometer capable of reading 96-well microplates (lag time 30.0 secs and read time 1.0 sec/well)
3. Multi and single channel pipettes and sterile pipette tips
4. Squirt bottle or automated microplate washer
5. Deionized or distilled water
6. Tubes to prepare standard or sample dilutions
7. Absorbent filter papers
8. 100 ml and 1 L volume graduated cylinders

Protocol

A. Preparation of sample and reagents

1. Sample

Isolate the test samples soon after collecting, analyze immediately or store at 4°C for up to 5 days. Otherwise, store at -20°C for up to one month or -80°C for up to two months to avoid loss of bioactivity. Avoid multiple freeze-thaw cycles.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 60 minutes. Centrifuge at approximately 1000 × g for 20 min. Analyze the serum immediately or aliquot and store at -20°C or -80°C.
- **Plasma:** Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 × g within 30 minutes of collection. Assay immediately or aliquot and store at -20°C. Avoid hemolysis and high cholesterol samples.
- **Urine:** Aseptically collect the first urine of the day (mid-stream) voided directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
- **Other biological fluids:** Centrifuge at approximately 1000 × g for 20 min to remove precipitant. Analyze immediately or aliquot and store at -20°C or -80°C.

Note:

» Please bring sample slowly to room temperature. Sample hemolysis will influence the result. Hemolyzed specimen should not be used.

» Samples must be diluted so that the expected concentration falls within the kit's range. Sample should be diluted in 0.01 mol/L PBS (PH=7.0-7.2). Serum/plasma samples require approximately a 4 fold dilution.

» If the sample are not indicated in the manual's applications, a preliminary experiment to determine the validity of the kit will be necessary.

» Fresh sample or recently obtained samples are recommended to prevent protein degradation and denaturalization that may lead to erroneous results.

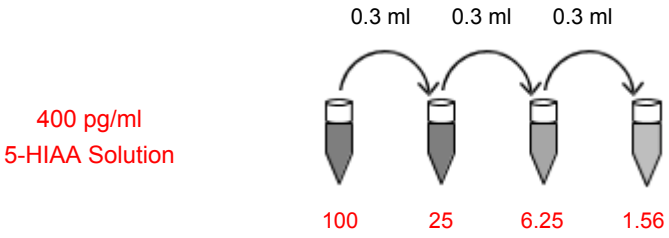
» Always use non-pyrogenic, endotoxin-free tubes for blood collection.

2. Wash buffer

Dilute the concentrated Wash buffer 30-fold (1/30) with distilled water (i.e. add 20 ml of concentrated wash buffer into 580 ml of distilled water).

3. Standard

Bring samples and all kit components to room temperature. Prepare the Standard with 1 ml of Standard Diluent buffer (kept for 10 min at room temperature) to make the 400 pg/ml Standard Solution. Allow the reconstituted standard to sit for 10 minutes with gentle agitation prior to carrying out the serial dilutions; avoiding foaming or bubbles. Label 4 tubes with 100 pg/ml, 25 pg/ml, 6.25 pg/ml, 1.56 pg/ml. Aliquot 0.7 ml of the Standard diluent buffer into each tube. Add 0.3 ml of 400 pg/ml standard solution into the 1st tube and mix thoroughly. Transfer 0.3 ml from 1st tube to 2nd tube, mix thoroughly, and so on.



4. Detection Reagent A and B Preparation

Centrifuge Detection Reagent A and B briefly before use. Detection Reagent A and B should be diluted 100-fold with the Diluent A and B and mixed thoroughly. They are sticky solutions, therefore pipette with a slow, smooth action to reduce volume errors. Please

discard after use.

5. Substrate working solution Preparation

Substrate A and B should be mixed with a ration of 99:1 respectively and mixed thoroughly. For example, prepare 1 ml of substrate working solution by mixing 0.99 ml of Substrate A and 0.01 ml of Substrate B.

B. Assay Procedure

Equilibrate the kit components and samples to room temperature before use. It is recommended to plot a standard curve for each test.

1. Set standard, test sample and control (zero) wells on the pre-coated plate and record their positions. It is recommended to measure each standard and sample in duplicate or triplicate. Add the solution at the bottom of each well without touching the side walls.
2. Add 50 µl of the diluted standards into the standard wells. Aliquot 50 µl Standard Diluent Buffer to the control (zero) well.
3. Add 50 µl of appropriately diluted sample into the test sample wells. Add the solution at the bottom of each well without touching the side wall. Shake the plate mildly to mix thoroughly.
4. Immediately aliquot 50 µl of Detection Reagent A working solution (if it appears cloudy mix gently until the solution is uniform) to each well. Shake the plate gently to mix thoroughly (a microplate shaker is recommended).
5. Seal the plate with a cover and incubate for 1 h at 37°C.
6. Remove the cover and discard the solution. Wash the plate 3 times with 1X Wash Buffer. Fill each well completely with Wash buffer (350 µl) using a multi-channel Pipette or autowasher (1-2 minute soaking period is recommended). Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean absorbent paper towels.
7. Aliquot 100 µl of Detection Reagent B working solution into each well, seal and incubate at 37°C for 30 min.
8. Repeat the aspiration/wash process 5 times as explained in step 6.
9. Aliquot 90 µl of TMB Substrate into each well. Seal the plate with a cover and incubate at 37°C for 10-20 min. Avoid exposure to light. The incubation time is for reference use only, the optimal time should be determined by end user. Do not exceed 30 min.
10. Add 50 µl of Stop solution into each well. There should be a color change to yellow. Gently tap the plate to ensure thorough mixing.
11. Measure the chemiluminescence signal in a microplate luminometer immediately.

For calculation, (the relative RLU) = (the RLU of each well) – (the RLU of Zero well). The standard curve can be plotted as the relative RLU of each standard solution (Y) vs. the respective concentration of the standard solution (X). Log-log curve fitting is recommended for data analysis. The 5-HIAA concentration of the samples can be interpolated from the standard curve.

Note: If the samples measured were diluted, multiply the dilution factor by the concentrations from interpolation to obtain the concentration before dilution.

C. Precautions

1. Before using the kit, centrifuge the tubes briefly to bring down the contents trapped in the lid.
2. Wash buffer may crystallize and separate. If this happens warm to room temperature and mix gently until the crystals are completely dissolved.
3. Avoid foaming or bubbles when mixing or reconstituting components. Prepare the Standard solutions within 15 min of starting the experiment. Please use the diluted Standard for a single assay procedure and discard after use. For each step in the procedure, total dispensing time for addition of reagents to the assay plate should not exceed 10 minutes.
4. It is recommended measuring each standard and sample in duplicate.
5. Do not let the wells uncovered for extended periods between incubation. Once reagents are added to the wells, avoid letting the strips dry as this can inactivate the biological material on the plate. Incubation time and temperature must be controlled.
6. Ensure plates are properly sealed or covered during incubation steps.
7. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.
8. Do not reuse pipette tips and tubes to avoid cross contamination.
9. Do not use expired components or components from a different kit.
10. Store the substrate A and B in the dark.

D. Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, medium and high levels of 5-HIAA were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, medium and high levels of 5-HIAA were tested on 3 different plates, 8 replicates in each plate.

CV (%) = (Standard Deviation / mean) × 100

Intra-Assay: CV<10%

Inter-Assay: CV<12%