5-Hydroxytryptamine (Serotonin) ELISA Detection Kit

Cat. No. KITG-041 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	This kit is for research use only and not for use in medical diagnosis. This ELISA Detection Kit is designed for the quantitative detection of 5-Hydroxytryptamine (5-HT, also known as Serotonin) in samples using a competitive enzyme-linked immunosorbent assay (ELISA) method. The assay utilizes a pre-coated microplate with 5-HT antibody. Samples, standards, and HRP-labeled competing antigen are added sequentially to the coated wells. After incubation and thorough washing, the substrate TMB is added for color development. TMB is converted to blue under the catalysis of peroxidase and then to yellow upon acidification. The intensity of color is inversely proportional to the concentration of 5-HT in the sample. The absorbance (OD value) is measured at 450 nm using a microplate reader, and sample concentration is calculated.
Description	This 5-Hydroxytryptamine (Serotonin) ELISA Detection Kit provides a competitive ELISA method for the quantitative detection of 5-HT in various biological samples including serum, plasma, cell culture supernatant, and tissue homogenates. The kit uses a pre-coated 96-well microplate with anti-5-HT antibody. The competitive assay format utilizes HRP-labeled competing antigen, with TMB as the chromogenic substrate. The assay has a sensitivity of 1.0 pg/mL and a detection range of 1.0-128 pg/mL. The kit is available in both 96-well and 48-well configurations and includes all necessary reagents for performing the assay.
Form	Liquid
Applications	Quantitative detection of 5-Hydroxytryptamine (Serotonin) in serum samples;

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	Quantitative detection of 5-HT in plasma samples (EDTA, citrate, or heparin anticoagulated); Quantitative detection of 5-HT in cell culture supernatants; Quantitative detection of 5-HT in tissue homogenates; Research on serotonin-related physiological and pathological processes
Notes	This kit is for research use only and not for use in medical diagnosis or human experimentation. Store the kit at 2-8°C. Before use, equilibrate to room temperature for 20 minutes. Crystals may form in the concentrated wash buffer stored at 4°C; this is normal. Warm in a water bath to completely dissolve crystals before use. Unused plate strips should be immediately returned to the sealed bag and stored at low temperature in dry conditions. According to the instructions, samples have already been diluted 5-fold; the final result must be multiplied by 5 to obtain the actual sample concentration. Strictly follow the time, volume, and sequence of incubation operations as specified in the instructions. All liquid components should be thoroughly mixed before use. Read the OD values within 15 minutes after adding the stop solution. The standard curve can be plotted using a 4-parameter equation (4PL) or using the microplate reader's built-in software. If samples are diluted, multiply by the corresponding dilution factor. Avoid repeated freeze-thaw cycles of samples. Ensure samples are uniformly and completely thawed at room temperature before testing.
Storage	Store at 2-8°C, protected from light and moisture. Valid for 6 months.
Shipping	Shipped with ice packs
Reaction conditions	Coating: Pre-coated with 5-HT antibody Incubation temperature: 37°C Incubation time: 60 min (after adding HRP-labeled competing antigen) Substrate incubation: 37°C, 15 min, protected from light Stop solution addition: 50 µL per well Measurement wavelength: 450 nm Reading time: Within 15 min after adding stop solution
Unit definition	Sensitivity: 1.0 pg/mL Detection range: 1.0-128 pg/mL Standard curve concentrations:

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	0, 8, 16, 32, 64, 128 pg/mL
Usage	Kit Components (96-well configuration): Pre-coated Microplate: 12 wells × 8 strips Standards (S0-S5): 0.3 mL × 6 tubes (Concentrations: 0, 8, 16, 32, 64, 128 pg/mL) Sample Diluent: 6 mL HRP-labeled Competing Antigen: 6 mL 20× Wash Buffer: 25 mL Substrate A: 6 mL Substrate B: 6 mL Stop Solution: 6 mL Plate Sealer: 2 sheets Instruction Manual: 1 copy Sealed Bag: 1 piece Kit Components (48-well configuration): Pre-coated Microplate: 12 wells × 4 strips Standards (S0-S5): 0.3 mL × 6 tubes (Concentrations: 0, 8, 16, 32, 64, 128 pg/mL) Sample Diluent: 3 mL HRP-labeled Competing Antigen: 3 mL 20× Wash Buffer: 15 mL Substrate A: 3 mL Substrate B: 3 mL Stop Solution: 3 mL Plate Sealer: 2 sheets Instruction Manual: 1 copy Sealed Bag: 1 piece Materials Required but Not Provided: Microplate reader (450 nm) High-precision pipettes and tips: 0.5-10 µL, 2-20 µL, 20-200 µL, 200-1000 µL 37°C incubator Distilled water Sample Collection, Processing, and Storage: 1. Serum: Use tubes without pyrogens and endotoxins. Avoid any cell stimulation during operation. After blood collection, centrifuge at 3000 rpm for 10 minutes to separate serum from red blood cells carefully and rapidly. 2. Plasma: Use EDTA, citrate, or heparin as anticoagulant. Centrifuge at 3000 rpm for 30 minutes and collect the supernatant. 3. Cell Culture Supernatant: Centrifuge at 3000 rpm for 10 minutes to remove particles and polymers. 4. Tissue Homogenate: Add appropriate amount of physiological saline to the tissue and homogenize. Centrifuge at 3000 rpm for 10 minutes and collect the supernatant. 5. Storage: If samples are not tested immediately after collection, aliquot according to single-use amount and store at -20°C. Avoid repeated freeze-thaw cycles. Thaw at room temperature and ensure samples are uniformly and completely thawed before testing. Reagent Preparation: Dilution of 20× Wash Buffer: Dilute with distilled water at 1:20 ratio, i.e., 1 part of 20× Wash Buffer plus 19 parts of distilled water. Plate Washing Methods: 1. Manual Washing: Empty the wells thoroughly, fill each well with wash buffer, incubate for 1 min, then empty the wells again. Pat dry on absorbent paper. Repeat this washing procedure 5 times. 2. Automatic Plate Washer: Inject 350

μL of wash buffer per well, soak for 1 min, wash 5 times. Assay Procedure: 1. Remove the required number of strips from the aluminum foil bag after equilibrating at room temperature for 20 min. Return the remaining strips to the sealed bag and store at 4°C. 2. Set up standard wells and sample wells. Add 50 μL of different concentration standards to the standard wells. 3. For sample wells, first add 10 μL of sample, then add 40 μL of Sample Diluent. Do not add anything to blank wells. 4. Except for blank wells, add 50 μL of HRP-labeled competing antigen to each standard well and sample well. Seal the wells with plate sealers. Incubate in a 37°C water bath or incubator for 60 min. 5. Discard the liquid, pat dry on absorbent paper. Fill each well with wash buffer, incubate for 1 min, discard the wash buffer, and pat dry on absorbent paper. Repeat this washing procedure 5 times (or use an automatic plate washer). 6. Add 50 μL of Substrate A and 50 μL of Substrate B to each well. Incubate at 37°C protected from light for 15 min. 7. Add 50 μL of Stop Solution to each well. Read the OD values of each well at 450 nm wavelength within 15 min. Data Analysis: 1. Read the OD values of each well at 450 nm wavelength within 15 min using a microplate reader. 2. Plot the standard curve with concentration as the X-axis and average OD450 value as the Y-axis. A 4-parameter equation (4PL) can be used to plot the standard curve. Alternatively, use the built-in plotting software of the microplate reader (such as SkanIt RE software for Thermo FC model). 3. Substitute the sample OD450 value into the standard curve to calculate the sample concentration. If the sample was diluted, multiply by the corresponding dilution factor. Kit Performance: 1. Accuracy: The correlation coefficient R value of standard linear regression with expected concentration is greater than or equal to 0.9900. 2. Sensitivity: The minimum detection concentration is less than 1.0 pg/mL. 3. Detection Range: 1.0-128 pg/mL. 4. Specificity: No cross-reactivity with other soluble structural analogs. 5. Reproducibility: Both intra-assay and inter-assay coefficients of variation are less than 15%.

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