Human HBsAg ayw ELISA Kit

Cat. No. KITG-104 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	The Human HBsAg ayw ELISA Kit is intended for the quantitative determination of human hepatitis B surface antigen ayw subtype (HBsAg ayw) in serum, plasma, and related samples. The assay uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) format. The kit contains 96 tests. The stated detection range is 1-50 IU/L.
Species	Human
Description	Assay Principle This kit uses a double-antibody sandwich enzyme-linked immunosorbent assay to measure human hepatitis B surface antigen ayw subtype (HBsAg ayw). Purified antibody is coated onto the microplate to form a solid-phase antibody. HBsAg ayw in standards or test samples binds to the coated antibody and subsequently binds to the HRP-labeled detection antibody, forming an antibody-antigen-HRP-labeled antibody complex. After thorough washing, TMB substrate is added for color development. TMB is converted to blue under the catalytic action of HRP and then turns yellow after addition of the acidic stop solution. Color intensity is positively correlated with the amount of HBsAg ayw in the sample. Absorbance is measured at 450 nm, and the human HBsAg ayw concentration is calculated from the standard curve. Assay Characteristics Kit size: 96T. Detection range: 1-50 IU/L. Detection format: Double-antibody sandwich ELISA.
Form	Kit
Applications	Quantitative measurement of human hepatitis B surface antigen ayw subtype (HBsAg

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	ayw) in serum, plasma, and related samples.
Notes	Kit Components 30× Concentrated Wash Solution: 20 mL × 1 bottle. Enzyme Conjugate: 6 mL × 1 bottle. Antibody-Coated Microplate: 12 wells × 8 strips. Sample Diluent: 6 mL × 1 bottle. Color Reagent A: 6 mL × 1 bottle. Color Reagent B: 6 mL × 1 bottle. Stop Solution: 6 mL × 1 bottle. Standard, 80 IU/L: 0.5 mL × 1 bottle. Standard Diluent: 1.5 mL × 1 bottle. Instruction Manual: 1 copy. Plate Sealing Film: 2 sheets. Resealable Bag: 1. Sample Requirements 1. After sample collection, perform extraction as soon as possible according to the relevant literature, and conduct the assay promptly after extraction. If the assay cannot be performed immediately, store samples at -20°C and avoid repeated freeze-thaw cycles. 2. Do not test samples containing NaN_3 because sodium azide inhibits horseradish peroxidase (HRP) activity. Precautions 1. After removing the kit from refrigerated storage, equilibrate it at room temperature for 15-30 minutes before use. If the antibody-coated microplate is not completely used after opening, place the remaining strips in the resealable bag for storage. 2. Crystals may form in the concentrated wash solution. Warm the solution in a water bath during dilution to help dissolve the crystals. This does not affect washing results. 3. Use a pipette for every dispensing step and calibrate it frequently to minimize experimental error. Complete each dispensing operation within 5 minutes whenever possible. For a large number of samples, use of a multichannel pipette is recommended. 4. Prepare a standard curve for every assay; duplicate wells are recommended. If the target concentration in a sample is too high, indicated by a sample OD greater than the OD of the first standard well, dilute the sample n-fold with Sample Diluent and repeat the assay. Multiply the calculated result by the total dilution factor, n × 5. 5. Use each plate sealing film only once to avoid cross-contamination. 6. Protect the substrate from light during storage. 7. Follow the instructions strictly. Interpret assay results according to the microplate-reader measurements. 8. Treat all samples, wash solution, and waste as potentially

	infectious materials. 9. Do not mix components from different kit lots.
Storage	Store the kit at 2-8°C. The stated shelf life is 6 months.
Reaction conditions	Standard Preparation The kit provides one vial of the original standard at 80 IU/L. Prepare the serial standards in small tubes as follows: 40 IU/L, Standard No. 5: Add 150 µL original standard to 150 µL Standard Diluent. 20 IU/L, Standard No. 4: Add 150 µL Standard No. 5 to 150 µL Standard Diluent. 10 IU/L, Standard No. 3: Add 150 µL Standard No. 4 to 150 µL Standard Diluent. 5 IU/L, Standard No. 2: Add 150 µL Standard No. 3 to 150 µL Standard Diluent. 2.5 IU/L, Standard No. 1: Add 150 µL Standard No. 2 to 150 µL Standard Diluent. Assay Procedure 1. Set up blank wells, standard wells, and test-sample wells. Do not add sample or Enzyme Conjugate to the blank wells; perform all other steps in the same manner. Add 50 µL of the appropriate standard to each standard well. For each test-sample well, first add 40 µL Sample Diluent and then add 10 µL test sample, giving a final 5-fold sample dilution. Dispense the liquid onto the bottom of each well, avoid touching the well wall, and gently shake to mix. 2. Cover the plate with sealing film and incubate at 37°C for 30 minutes. 3. Dilute the 30× Concentrated Wash Solution 30-fold with distilled water before use. 4. Carefully remove the sealing film, discard the liquid, and shake the plate dry. Fill each well with wash solution, allow it to stand for 30 seconds, discard the wash solution, and repeat for a total of 5 wash cycles. Tap the plate dry. 5. Add 50 µL Enzyme Conjugate to each well except the blank wells. 6. Cover the plate and incubate at 37°C for 30 minutes as described above. 7. Wash the plate 5 times as described above. 8. Add 50 µL Color Reagent A to each well, followed by 50 µL Color Reagent B. Gently shake to mix and incubate at 37°C protected from light for 10 minutes. 9. Add 50 µL Stop Solution to each well to stop the reaction. The color changes immediately from blue to yellow. 10. Zero the microplate reader with the blank well and measure the absorbance of each well sequentially at 450 nm. Complete the measurement within 15 minutes after adding Stop Solution.

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Unit definition	Result Calculation Use the standard concentration as the horizontal axis and the OD value as the vertical axis to plot a standard curve. Determine the corresponding sample concentration from the sample OD value and multiply by the dilution factor. Alternatively, calculate a linear-regression equation from the standard concentrations and OD values, substitute the sample OD value into the equation, calculate the sample concentration, and then multiply by the dilution factor to obtain the actual sample concentration. The routine sample-well setup produces a 5-fold final dilution. If a sample is additionally diluted n-fold before testing, multiply the concentration calculated from the standard curve by the total dilution factor, n × 5.
Usage	For Research Use Only.

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