USER GUIDE

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HBsAgGi ELISA Kit (96 Test)

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CONTENTS

1 Product description

The HBsAgGi ELISA Kit is an enzyme-linked immunosorbent assay for the quantitative detection of HBV particles containing *O*-glycosylated M-HBsAg. For Research Use Only. Not for use in diagnostic procedures.

2 Reagents for HBsAgGi ELISA

2.1 Materials included in this kit

1) HBsAgGi antibody coated plate：96 well (8-well strip x 12)
2) Standard M-HBsAg (800 ng/mL)：1 mL (2 mL tube)
3) Dilution Buffer：24 mL (12 mL x 2 bottles)
4) HRP-labeled HBsAgGi antibody：11 mL
5) 20X Wash Buffer：50 mL
6) TMB Substrate：12 mL
7) Stop Solution (0.2 M Sulfuric acid)：12 mL
8) Plate seal：3
9) User Guide

2.2 Materials and equipment required but not provided

1) Plate reader：450 nm
2) Plate washer
3) Plate shaker (If available)
4) Reagent reservoir
5) Micropipette (20 µL, 200 µL, 1000 µL)
6) Multi-channel pipette
7) Disposable microtubes and pipet tips
8) Distilled water

3 Summary

The HBs antigen that constitutes the envelope of HBV particles consists of three glycoproteins of different sizes (S-HBs antigen, M-HBs antigen, and L-HBs antigen), and infectious HBV particles with HBV DNA or HBV RNA have all HBs antigens. On the other hand, the blood of HBV-infected patients contains many subviral particles that are not infectious, and the subviral particles mainly consist of S-HBs antigen. The anti-HBV surface antigen glycosylated isomeric antibody (HBsAgGi) used in this kit is specific for genotype C and binds mainly to infectious HBV particles.

4 Principle

This product is a kit for HBV measurement by sandwich ELISA method. An anti-HBV surface antigen glycosylation isomer antibody (HBsAgGi) is adsorbed onto microwells. HBV particles and standards can be captured by HBsAgGi antibody (STEP 1). After washing, the HBV particles captured on the ELISA microwells will be further reacted with the peroxidase conjugated antibody, HRP-labeled HBsAgGi (STEP 2). After further washing, HRP substrate (TMB substrate) is added into the well. After the incubation, the reaction is stopped by the addition of Stop solution and the absorbance at 450nm is measured by a plate reader (STEP 3). The M-HBsAg protein concentration in the sample will be calculated by using a calibration curve prepared by measuring the standard material (M-HBsAg protein in the kit) with different concentrations.

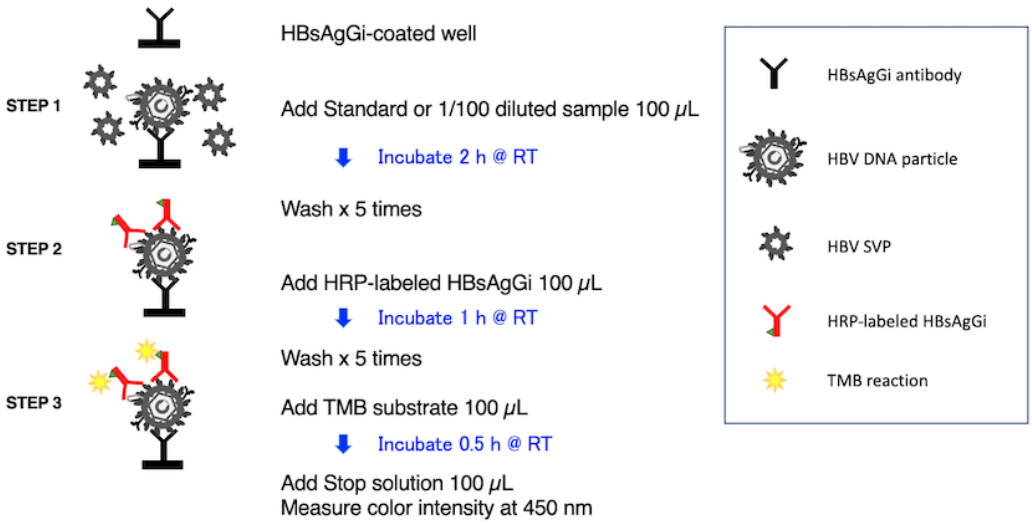


Figure 1 Procedures of HBsAgGi ELISA kit

5 Procedure

5.1 Preparation

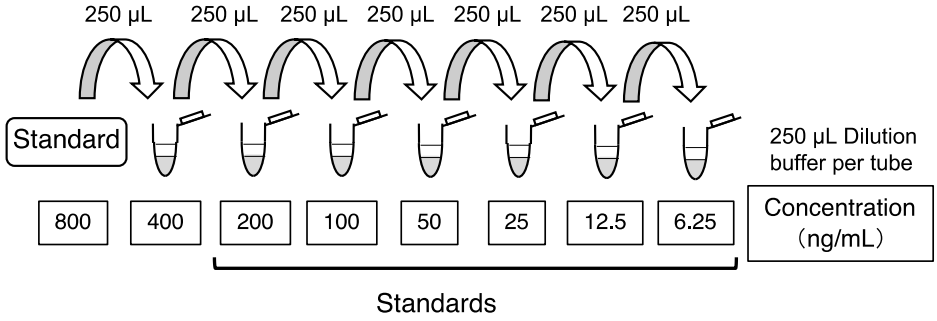
1) Cell culture supernatant, serum, and plasma were tested with this assay. Serum samples should be collected according to the usual method and assayed within 1 week at 4°C after collection. If stored longer, samples should be aliquoted and must be stored frozen at -20°C or below and avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature. Samples containing a visible precipitate must

be clarified before use for the assay.

2) Prepare 1X washing solution by mixing 50 mL of 20X Wash Buffer with 950 mL of purified water. After mix well, transfer to a clean bottle and store at 4–30°C. The 1X washing solution is stable for 30 days.

5.2 Preparation of standards

Prepare seven microcentrifuge tubes, labeled 400 ng/mL, 200 ng/mL, 100 ng/mL, 50 ng/mL, 25 ng/mL, 12.5 ng/mL, and 6.25 ng/mL. Add 250 µL of Dilution Buffer to each tube. Spin down the tube of Standard M-HBsAg (800 ng/mL) in the kit. Take 250 µL of the Standard M-HBsAg and add it to the 400 ng/ml tube and mix. Next, take 250 µL from the 400 ng/ml tube and add it to the 200 ng/mL tube and mix. Make a 2-fold serial dilution to 6.25 ng/mL in the same manner.



5.3 Sample dilution method

Serum samples should be diluted 100-fold with Dilution Buffer; for duplicate assays (recommended), add 217.8 µL of Dilution Buffer to a dilution test tube, add 2.2 µL of sample, and mix.

5.4 Preparation of reagents

1) Antibody for detection: HRP-labeled HBsAgGi antibody should be used without dilution. For the remainder of the HRP-labeled HBsAgGi antibody, close the tube tightly and keep storing at 2-8°C.
2) TMB Substrate: Remove from refrigerator and allow to come to room temperature. It can be used as is.
3) Stop Solution: Remove from the refrigerator and let it come to room temperature. It can be used as is.

5.5 Measuring protocol

1) Determine the number of microwell strips required for measuring samples and number of wells for standards and blanks. Each sample, standard, and blank should be assayed in duplicate (recommended). Remaining microwell strips should be returned into the provided bag, which must be carefully resealed to avoid moisture absorption and stored at 2-8°C
2) Standards: Add 100 µL of the Dilution Buffer (blank standards) in two wells of the microwell strips as the blanks. In the same way, add 100 µL of each standard ranging from 6.25 ng/mL to 200 ng/mL to two wells (100 µL/well).
3) Samples: Add 100 µL of samples 100-fold diluted with Dilution Buffer into two wells (100 µL/well).
4) First incubation: After all additions have been made, seal the microwell strips and incubate for 2 hours at room temperature. If available, gently shake the microwell strips (approximately 400 rpm/min).
5) Wash: Remove the plate seal gently and empty wells. Wash microwell strips 5 times with Wash Buffer (350 µL/well).
6) HRP-labeled HBsAgGi: Add 100 µL of HRP-labeled HBsAgGi to all wells, including the blank wells.
7) Second incubation: Seal the microwell strips and incubate for 1 hour at room temperature. If available, gently shake the microwell strips (approximately 400 rpm/min).
8) Wash: Remove the plate seal gently and empty wells. Wash microwell strips 5 times with Wash Buffer (350 µL/well).
9) HRP reaction: Prepare TMB Substrate in a reservoir with enough amount for the assay. By using a multi-channel pipette, add 100 µL of TMB Substrate to all wells and incubate the strips at room temperature up to 30 min. The color development of the well should be monitored and the reaction should be stopped at the appropriate timing.
   The reaction will present blue coloration. Stop the reaction of the wells with blank being clear, around 25 ng/mL being light blue, and 200 ng/ml being dark blue.
10) Stop reaction: Add 100 µL of the Stop Solution to each well from the reservoir with enough amount for the assay by using a multi-channel pipette. Mix the solution to stop the reaction by gently shaking the microwell strips. The color of the wells will change from blue to yellow after the addition of the stop solution. Note: The Stop Solution is 0.2 M H_2SO_4, please use with caution.

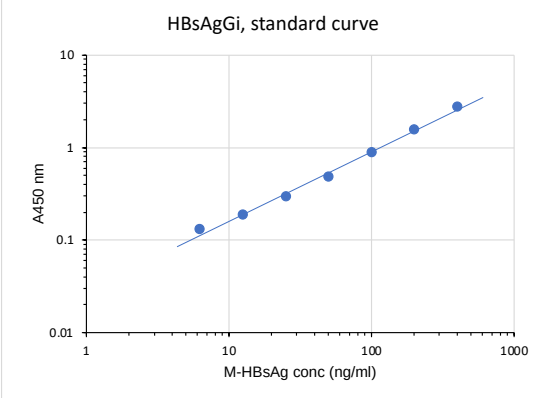
11) Measurement: After stopping the reaction, read the absorbance of each microwell at 450 nm with a plate reader within 15 minutes. Optionally, read the absorbance at 620 nm as the reference wave length. Determine the absorbance of both samples and standards including blanks.

NOTE: In case of incubation using a shaker, the obtained values may be higher than the value obtained without shaking.

6 Calculation of the concentration

1) Calculate the average absorbance of each concentration of the standard M-HBsAg reactive with HBsAgGi antibody.
2) Prepare a calibration curve by taking the concentration of the standard on the horizontal axis and the absorbance of 450 nm on the vertical axis of the logarithmic graph.
3) Calculate the concentration of M-HBsAg from the absorbance readings of each sample according to the above calibration curve graph.
4) Quantification cannot be performed outside the range of the calibration curve. If the measured value is near the upper limit of the calibration curve (O.D. 450 value is higher than 2.0), measure again after dilution to obtain the amount of M-HBsAg.
5) Example of standard curve of M-HBsAg is shown.

Concentration of standard (ng/mL)	O.D. 450
0 (blank)	0.078
6.25	0.132
12.5	0.188
25	0.294
50	0.482
100	0.890
200	1.565
400	2.747



7 Performance characteristics

7.1. Sensitivity

The limit of detection of M-HBsAg was determined to be 0.89 ng/mL (mean plus 3 standard deviations obtained from 10 independent measurements).

7.2. Reproducibility (Intra-assay)

Intra-assay reproducibility was determined by assaying the serum samples at 6 replicates. Coefficient of variation (C.V.) value on the same plate using a known concentration of 3 samples is less than 10%.

Sample	Serum 1	Serum 2	Serum 3
Number of determinations	6	6	6
Mean (ng/mL)	110.4	45.0	89.2
C.V. (%)	1.82	2.52	2.65

8 Precautions

8.1 Precautions for handling (hazard prevention)

1) Serum specimens and samples should be handled with the caution that they are infectious. In order to avoid the risk of infection, protective measures such as safety cabinets, gloves, lab coats, goggles, and masks should be taken during measurement.
2) This kit contains sodium azide as a preservative. If the reagent is accidentally put into the eyes or mouth, or comes in contact with the skin, first aid measures such as rinsing thoroughly with water should be taken, and medical attention should be sought as necessary.
3) Since this kit contains sulfuric acid as a reaction stop solution, if it is accidentally put into the eyes or mouth, or adheres to the skin, take first aid measures such as rinsing thoroughly with water. If necessary, see a medical doctor.

8.2 General precautions

1) The designated reference material (recombinant Standard M-HBsAg) and diluted standards should be used for calibration curve preparation.
2) The specified sample diluent should be used for dilution of samples.
3) A calibration curve should be prepared for each sample measurement.
4) The reagent should be used immediately after opening the kit and stored at 2-8°C with the lid closed.
5) The reagent should be stored under the specified conditions and should not be used after the expiration date.
6) This kit is for research use only and should not be used for any purpose other than those specified.

8.3 Precautions for disposal

1) Serum specimens and samples should be handled as potentially infectious, and precautions should be taken regarding the disposal of used tips, plates, and liquid waste.
2) Chips used for specimens should be sterilized with sodium hypochlorite (soaked in 1% effective concentration for at least 1 hour). Chips, plates and waste solution should be sterilized by autoclaving (121°C, 20 minutes or longer).
3) Waste solution should be disposed of in accordance with the Water Pollution Control Law and other relevant regulations.
4) Since the Dilution buffer contains sodium azide as a preservative, the liquid waste should be wasted by a specialized waste disposal company
5) Waste materials (used plastic products) should be disposed of as chemical adherents by a specialized waste disposal company.
6) Specimens should be handled in a safety cabinet, but if they are accidentally scattered or contaminated, wipe them off with paper towels, and then sterilize them by wiping with sodium hypochlorite (effective concentration: 1%).

9 Storage and stability

All kit components except Stop Solution must be stored at 2-8°C. All reagents are stable for 12 months after manufacturing when stored at the indicated conditions.

10 References

1. Schmitt et al. Structure of pre-S2 *N*- and *O*-linked glycans in surface proteins from different genotypes of hepatitis B virus. (2004) J Gen Virol 85:2045-2053.
2. Dobrica et al. *N*-Glycosylation and *N*-Glycan Processing in HBV Biology and Pathogenesis. (2020) Cells 9:1404.
3. Angata et al. *O*-glycosylated HBsAg peptide can induce specific antibody neutralizing HBV infection. (2021) Biochim Biophys Acta Gen Subj. 1866:130020

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