TECHNICAL MANUAL

Human HBsAg (Hepatitis B Virus Surface Antigen) ELISA Kit

- **SKU CODE:** HUFI08043
- **SIZE:** 96T
- **DETECTION PRINCIPLE:** Qualitative Sandwich ELISA Kit
- **RUO:** Research-Use-Only

Revised: 11/26

Human HBsAg (Hepatitis B Virus Surface Antigen) ELISA Kit

Please read entire manual carefully before starting experiment. DO NOT mix reagents and use reagents from different kits or batches to prevent assay failure.

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1. Key features and Sample Types

Aliases:

HBsAg; Hepatitis B Virus Surface Antigen; HBV Surface Antigen

Detection method:

Sandwich (Qualitative ELISA)

Sample Type:

Serum, Plasma

Reactivity:

Human

2. Storage & Expiry

Assay Genie ELISA Kits are shipped on ice packs. Please store this unopened ELISA Kit at 2-8°C. For opened kits, store individual components as described in the Kit Contents section. Date of expiration will be on the ELISA Box label.

3. Description and Principle

The Assay Genie Human HBsAg (Hepatitis B Virus Surface Antigen) ELISA Kit enables qualitative detection of HBsAg in human serum and plasma samples.

This kit is based on sandwich enzyme-linked immunosorbent assay technology. HBsAb is pre-coated onto 96-well plates. The test samples are added to the wells, followed by Biotin-conjugated HBsAb. If HBsAg is present in the samples, an HBsAb - HBsAg - Biotin-HBsAb complex is formed. After incubation, the unbound components are washed away and HRP-Streptavidin is added. Following a further incubation and wash to remove unbound components, TMB substrate is used to visualize the HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue product that changes to yellow upon addition of the acidic stop solution.

The optical density (OD) of the developed color is measured at 450 nm using a microplate reader, with a selected reference wavelength within 650 nm. The results are interpreted qualitatively against a cutoff value calculated from the Negative Control (see Data Analysis).

This dual function kit includes validated Bradford Reagent to quantify total protein concentration for accurate sample normalization.

4. Kit Contents

The sealed kit can be stored at 2-8°C. The storage condition for the opened kit is specified in the table below. Each kit contains reagents for 96 assays including:

No	Component Name	Size (96T)	Storage
1	Micro ELISA Plate (Dismountable)	12 × 8	2-8°C / -20°C
2	HBsAg Positive Control	1 ml × 1	2-8°C
3	HBsAg Negative Control	1 ml × 1	2-8°C
4	Biotin-HBsAb	10 ml × 1	2-8°C (Avoid Direct Light)
5	HRP-Streptavidin	10 ml × 1	2-8°C (Avoid Direct Light)
6	Sample Dilution Buffer	20 ml × 1	2-8°C
7	TMB Substrate	10 ml × 1	2-8°C (Avoid Direct Light)
8	Stop Solution	10 ml × 1	2-8°C
9	Wash Buffer (25X)	30 ml × 1	2-8°C
10	Plate Sealer	3 pieces	-
12	Bradford Reagent	1 vial (2 ml)	RT
11	Technical Manual	1 copy	-

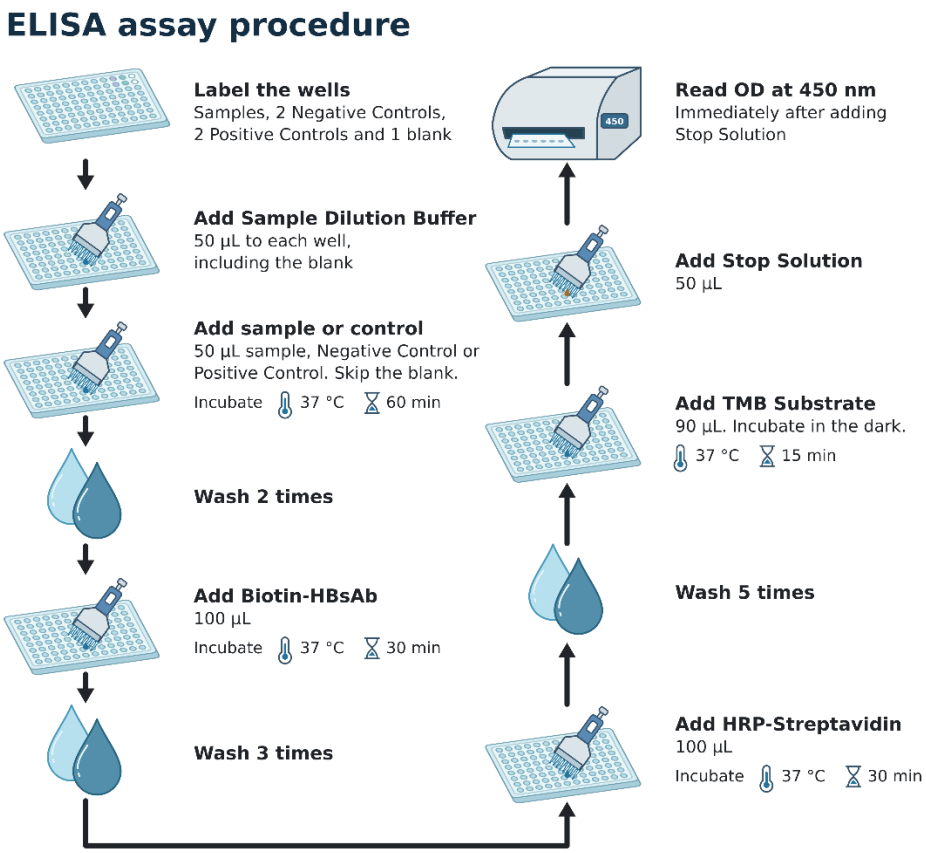
Additional materials required:

1. Microplate reader (wavelength: 450 nm).
2. 37°C incubator.
3. Automated plate washer.
4. Precision single and multi-channel pipettes and disposable pipette tips.
5. Clean tubes and Eppendorf tubes.
6. Deionized or distilled water.
7. Absorbent paper.

5. Precautions

- This kit is for research purposes only and not for diagnostics or therapeutic uses.
- Store all components as listed in this manual. Do not use the ELISA Kit after its expiration date.
- Allow all reagents and samples to reach room temperature before use.
- After opening and before use, keep the plate dry.
- Before using the kit, spin down the tubes to bring all components to the bottom of the tubes.
- Store TMB reagents protected from light.
- Washing is very important; insufficient washing can easily cause false positives and high background.
- Duplicate wells are recommended for both control and sample testing.
- Do not let the microplate wells dry during the assay, as a dry plate will inactivate the active components on the plate.
- Do not reuse tips and tubes to avoid cross contamination.
- Do not mix reagents from different kits or batches. Do not mix reagents from other manufacturers.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

6. Assay Summary



7. Sample Preparation

The procedures outlined in this document are provided as general recommendations for sample preparation in ELISA assays. Due to the variability of biological samples and specific assay requirements, users are advised to optimize protocols based on their own experimental conditions.

Note: For information regarding validation data in specific samples, please contact our Technical Support Team at techsupport@assaygenie.com.

General Considerations

Isolate the test samples soon after collection and analyze immediately (within 2 hours), or aliquot and store at -20°C for long-term storage. Avoid multiple freeze-thaw cycles.

- **Serum:** Allow the blood to coagulate at room temperature (about 1 hour). Centrifuge at approximately 1000 × g for 15 min. Analyze the serum immediately or aliquot and store at -20°C.
- **Plasma:** Collect plasma using heparin or EDTA as the anticoagulant. Centrifuge for 15 min at 1500 × g, 2-8°C, within 30 min of collection. To eliminate the platelet effect, further centrifugation for 10 min at 10000 × g, 2-8°C, is suggested. Analyze immediately or aliquot and store frozen at -20°C.

Note: Samples used within 5 days can be stored at 2-8°C; otherwise, they must be stored at -20°C, -80°C or in liquid nitrogen to avoid loss of biological activity and contamination. Avoid multiple freeze-thaw cycles. Hemolytic samples are not suitable for this test.

Other Sample Types: For information on other sample types, please contact our Tech Support Team at techsupport@assaygenie.com.

7.1. Protein Quantification (Optional)

To quantify total protein levels, use the Bradford Reagent included in this kit. Visit Bradford Protein Assay Protocol to view the full protocol.

8. Reagent Preparation

Manual Washing

Discard the solution from the plate without touching the sides of the wells. Tap the plate firmly onto absorbent filter paper or another absorbent material. Completely fill each well with 350 µl of wash buffer and let it soak for 1 to 2 minutes. Then aspirate the contents from the plate and tap it again onto absorbent filter paper or other absorbent material.

Automated Washing

Aspirate all wells, then wash the plate with 350 µl of wash buffer per well. After the final wash, invert the plate and tap it firmly on absorbent filter paper or another absorbent material. It is recommended to set the washer with a 1-minute soak time.

Note: Set the height of the needles; be sure the fluid can be taken up completely.

Reagent Preparation

Bring all reagents and samples to room temperature (18 - 25°C) before use. For repeated assays, please use only the strips required and store remaining reagents at the appropriate temperatures.

Wash Buffer: Dilute 30 ml of Concentrated Wash Buffer (25X) to 750 ml of Wash Buffer with deionized or distilled water. Crystals that form in the concentrated wash buffer can be dissolved by placing the solution in a water bath at 40°C until fully dissolved (do not exceed 50°C). Mix well before use.

9. Assay Procedure

1. **Plate Setup:** Label the sample wells, 2 Negative Control wells, 2 Positive Control wells and 1 blank well.
2. **Dilution Buffer:** Add 50 µl Sample Dilution Buffer to each well.
3. **Sample and Control Loading:** Add 50 µl of sample, Negative Control and Positive Control to the appropriate wells (except the blank well) and gently tap the plate to ensure thorough mixing.
4. **First Incubation:** Seal the plate with a cover and incubate at 37°C for 60 min.
5. **Washing:** Remove the cover and wash the plate twice without soaking.
6. **Biotin-HBsAb Addition:** Add 100 µl Biotin-HBsAb to each well. Seal the plate with a cover and incubate at 37°C for 30 min.
7. **Washing:** Wash the plate three times, allowing the wash buffer to soak for 1 min each time.

8. **HRP-Streptavidin Addition:** Add 100 µl HRP-Streptavidin to each well. Seal the plate with a cover and incubate at 37°C for 30 min.
9. **Washing:** Remove the cover and wash the plate 5 times with Wash Buffer, allowing the wash buffer to stay in the wells for 1 minute each time.
10. **Color Development:** Add 90 µl of TMB Substrate to each well. Cover the plate and incubate at 37°C in the dark for 15 min. A blue color should develop in the Positive Control wells, while Negative Control wells should show no obvious color.
11. **Stop Reaction:** Keep the liquid in the wells after color development. Add 50 µl of Stop Solution to each well and mix thoroughly. The color should immediately change to yellow.
12. **OD Measurement:** Read the O.D. absorbance at 450 nm in a microplate reader immediately after adding the Stop Solution (use the blank well to set zero).

10. Data Analysis

Assay validity

If the Negative Control is > 0.1, or the Positive Control is ≤ 0.4, the test is regarded as invalid.

Calculation of the Cutoff Value

$$\text{Cutoff Value} = NCx \times 2.1$$

NCx: Mean absorbance of the Negative Control. When NCx < 0.05, calculate as 0.05.

Determination of results

Samples with absorbance values ≤ Cutoff Value are NON-REACTIVE and are considered NEGATIVE for HBsAg. Samples with absorbance values > Cutoff Value are considered POSITIVE for HBsAg.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.