

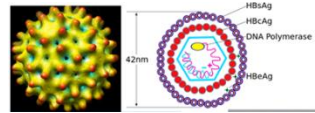
## INTENDED USE

The Alpha Diagnostics Int'l Hepatitis B Surface Antigen (HBsAg) ELISA Kit is a sandwich immunoassay for the quantification of HBsAg, recombinant or native, in cell culture, bioprocessing solutions, and/or in other appropriately qualified samples from tissue fluids (e.g., blood, saliva, mucosa). The assay is not intended for the diagnosis of Hepatitis B infection.

## GENERAL INFORMATION

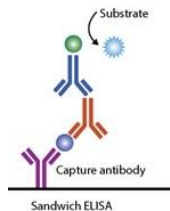
Hepatitis B is an infectious disease caused by hepatitis B virus (HBV). Hepatitis, the acute illness, inflames the liver, causing jaundice, vomiting and (rarely) death. Chronic hepatitis B, however, can cause cirrhosis and liver cancer – a fatal disease. Although viral replication occurs in the liver, HBV spreads to the blood where virus-specific antigens and antibodies may be found in the infected host. Blood tests for these antigens and antibodies are used to diagnose the infection. Acute and chronic hepatitis B can be prevented by vaccination.

HBV is divided into four major serotypes (adr, adw, ayr, ayw) based on antigenic epitopes presented on its envelope proteins, and into eight genotypes (A-H) according to overall nucleotide sequence variation of the genome. Genotypes differ by at least 8% of their sequences, differences which affect severity of disease and response to treatment and possibly vaccination.



The hepatitis B surface antigen (HBsAg or Australia antigen) is the first detectable viral antigen to appear during infection, and is most frequently used to screen for the presence of infection. HBsAg is also the basis for several recent vaccines, which use synthetic recombinant HBsAg and contain no blood products. Therefore, they cannot cause HBV infection, a problem with the original vaccine prepared from plasma from patients with long-term HBV infection. Following vaccination, HBsAg may be detected in serum for several days. These vaccines have provided protection for 85-90% of individuals. Patients who developed antibodies against HBsAg (anti-HBsAg seroconversion) are usually considered non-infectious. HBsAg detection by immunoassay is used in blood screening, to establish a diagnosis of hepatitis B infection in the clinical setting (in combination with other disease markers) and to monitor antiviral treatment.

## PRINCIPLE OF THE TEST



The HBsAg ELISA kit is based on the binding of HBsAg in samples to two antibodies, one immobilized on the microtiter wells, and the other conjugated to horseradish peroxidase (HRP) enzyme. After a washing step, chromogenic substrate is added and color is developed by the enzymatic reaction of HRP on the TMB substrate, which is directly proportional to the amount of HBsAg present in the sample.

Stopping Solution is added to terminate the reaction, and absorbance at 450nm is then measured using an ELISA microtiter well reader. The concentration of HBsAg in samples and control is calculated from standard curve containing known concentrations of HBsAg.

## KIT CONTENTS

The microtiter well plate and all other reagents, if unopened, are stable at 2-8°C until the expiration date printed on the box label. Stabilities of the working solutions are indicated under Reagent Preparation.

**To Be Reconstituted:** Store as indicated.

| Component   | Preparation Instructions  |
|---|---|
| <b>Sample Diluent Concentrate (20x)</b><br>Cat. No. SD-20T, 10ml              | Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as <b>Working Sample Diluent</b> and store at 2-8°C until the kit lot expires or is used up.  |
| <b>Wash Solution Concentrate (100x)</b><br>Cat. No. WB-100, 10ml              | Dilute the entire volume 10ml + 990ml with distilled or deionized water into a clean stock bottle. Label as <b>Working Wash Solution</b> and store at ambient temperature until kit is used entirely.   |
| <b>Anti-HBsAg - HRP Conjugate Concentrate (100x)</b><br>Part No. 4114, 0.15ml | Peroxidase conjugated anti-HBsAg in buffer with protein, detergents and antimicrobial as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of <b>Working Sample Diluent</b> is sufficient for 1 8-well strip. Use within the working day and discard. Return 100X to 2-8°C storage. |

**Ready For Use:** Store as indicated on labels.

| Component                                      | Part  | Amt                | Contents   |
|--|-------|--------------------|--|
| <b>Anti-HBsAg Microwell Strip Plate</b>        | 4111  | 8-well strips (12) | Coated with HBsAg viral antigens, and post-coated with stabilizers.  |
| <b>HBsAg Standards</b>                         |       |                    |  |
| 1 ng/ml  | 4113B | 0.65 ml            | Five (5) vials, each containing purified recombinant HBsAg with designated concentrations; diluted in buffer with protein, detergents and non-azide antimicrobials as stabilizers. |
| 3 ng/ml  | 4113C | 0.65 ml            |  |
| 6 ng/ml  | 4113D | 0.65 ml            |  |
| 12 ng/ml                                       | 4113E | 0.65 ml            |  |
| 20 ng/ml                                       | 4113F | 0.65 ml            |  |
| <b>Positive Control [HBsAg] range on label</b> | 4112  | 0.65 ml            | HBsAg of stated concentration range; diluted in buffer with protein, detergents and non-azide antimicrobials as stabilizers.   |
| <b>TMB Substrate</b>                           | 80091 | 12 ml              | Chromogenic substrate for HRP containing TMB and peroxide.   |
| <b>Stop Solution</b>                           | 80101 | 12 ml              | 1% sulfuric acid.  |

### Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml. A multi-channel pipettor is recommended.
- Disposable glass or plastic 5-15ml tubes for diluting samples and Antibody HRP Concentrate.
- Graduated cylinder to dilute Wash Concentrate; 0.2 to 1L.
- Stock bottle to store diluted Wash Solution; 200ml to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- Microwell plate reader at 450 nm wavelength.

## PRECAUTIONS AND SAFETY INSTRUCTIONS

Standards, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains 1% sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water. MSDS for TMB, sulfuric acid and BND can be requested or obtained from the ADI website: [http://4adi.com/commerce/info/showpage.jsp?page\\_id=1060&catogory\\_id=2430&visit=10](http://4adi.com/commerce/info/showpage.jsp?page_id=1060&catogory_id=2430&visit=10)

## ASSAY DESIGN AND SET-UP

### Sample Collection and Handling

Culture medium, bioprocessing preparations, serum and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference (See Limits of the Assay, page 6). For **serum**, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature.

For all samples, clarify by centrifugation and/or filtration prior to dilution in Sample Diluent. If samples will not be assayed immediately, store refrigerated for up to a few weeks, or frozen for long-term storage.

### Assay Validation

Validate the performance of the sample antigen and matrix in the assay system for recovery and parallelism (see Limits of the Assay, page 6), as follows:

**Recovery** – a measure of the interference of the sample matrix (diluent effect) in providing accurate quantitation of the sample HBsAg relative to the Standard curve.

Prepare and run a series of dilutions of the sample antigen (concentrations that will fall within the Standard range) in Working Sample Diluent to determine the dilutions that give consistent and accurate quantitation. For most buffer solutions a minimum 5-fold sample dilution is usually sufficient. Serum and plasma require at least a 20-fold dilution to obtain consistent quantitation or complete antigen recovery.

**Parallelism** – dilutions of the sample should read equivalent values from the top and bottom of the Standard curve to provide good assay precision.

Prepare a dilution series of the sample antigen that gives complete recovery and falls within the full range of the Standard curve. Sample readings from the upper and lower regions of the curve should differ by less than 25%.

### Plate Set-up

Bring all reagents to room temperature (18-30° C) equilibration (at least 30 minutes).

- Determine the number of wells for the assay run. Duplicates are recommended, including 10 Standard wells and 2 wells for each sample and control to be assayed.
- Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
- Add 200-300ul Working Wash Solution to each well and let stand for about 5 minutes. Aspirate or dump the liquid and pat dry on a paper towel before sample addition.

## Assay Procedure

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

### 1. 1<sup>st</sup> Incubation [100ul – 60 min; 4 washes]

- Add 100ul of calibrators, samples and controls each to pre-determined wells.
- Tap the plate gently to mix reagents and incubate for 60 minutes.
- Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility.

### 2. 2<sup>nd</sup> Incubation [100ul – 30 min; 5 washes]

- Add 100ul of diluted Anti-HBsAg HRP Conjugate to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 2.

### 3. Substrate Incubation [100ul – 15 min]

- Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.
- Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.

Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

### 4. Stop Step [Stop: 100ul]

- Add 100ul of Stop Solution to each well.
- Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

### 5. Absorbance Reading

- Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.
- Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.

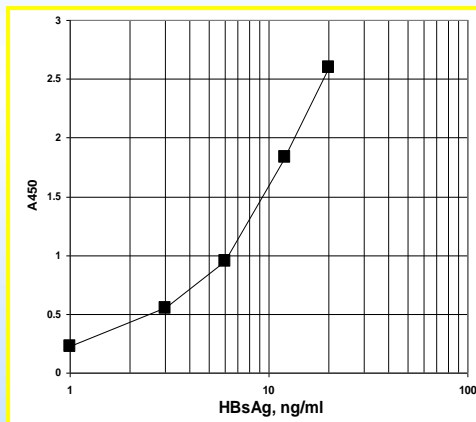
## CALCULATION OF RESULTS

- The results may be calculated using any immunoassay software package. The four-parameter curve-fit is recommended. If software is not available, HBsAg concentrations may be determined as follows:
- Calculate the mean OD of duplicate samples.
- On graph paper plot the mean OD of the standards (y-axis) against the concentration (ng/ml) of HBsAg (x-axis). Draw the best fit curve through these points to construct the standard curve. A point-to-point construction is most common and reliable.
- The HBsAg concentrations in unknown samples and controls can be determined by interpolation from the standard curve.
- Multiply the values obtained for the samples by the dilution factor of each sample.
- Samples producing signals higher than the 20 ng/ml standard should be further diluted and re-assayed.

### Typical Results:

| Wells  | Calibrators & Samples      | A450 nm | ng/ml |
|--------|----------------------------|---------|-------|
| A1, A2 | Diluent Blank              | 0.10    | 0     |
| B1, B2 | 1 ng/ml <b>Calibrator</b>  | 0.23    | 1     |
| C1, C2 | 3 ng/ml <b>Calibrator</b>  | 0.55    | 3     |
| D1, D2 | 6 ng/ml <b>Calibrator</b>  | 0.95    | 6     |
| E1, E2 | 12 ng/ml <b>Calibrator</b> | 1.84    | 12    |
| F1, F2 | 20 ng/ml <b>Calibrator</b> | 2.60    | 20    |
| G1, G2 | Sample [Diluted 1:100]     | 1.41    | 8.6   |

Calculated: 100-fold dilution x 8.6 ng/ml = **860 ng/ml** in serum



## PERFORMANCE CHARACTERISTICS

### Specificity

The antibodies used in this kit are specific for the surface antigen of hepatitis B (HBsAg), including native antigen from plasma of human carriers, and recombinant HBsAg derived from E. coli cultures, as used for the kit Standards, or from yeast cultures, as used in vaccines provided by Merck (Recombivax HB) and GlaxoSmithKline (Engerix B); see Limits of the Assay, below.

### Precision

Samples containing low, medium and high concentrations of HBsAg were assayed multiple times in the same assay (n=10) to provide within-assay precision, and as duplicates in multiple assays (n=5) to obtain between-assay reproducibility. Coefficients of variation were calculated for the concentrations using a point-to-point curve-fitting program.

HBsAg concentrations were measured with good within-assay (2.5 to 4.1 %CV) and between-assay (4.3 to 9.2 %CV) reproducibility.

| Sample        | HBsAg ng/ml | Intra-assay %CV | Inter-assay %CV |
|---------------|-------------|-----------------|-----------------|
| High Conc'n   | 12.7        | 2.5             | 9.2             |
| Medium Conc'n | 7.6         | 2.6             | 4.3             |
| Low Conc'n    | 3.0         | 4.1             | 6.8             |

### Specificity of the HBsAg detection

HBsAg (Ad, native, human plasma), HBsAg (Ad/Ay, recombinant, P. Pastoris) were detected very well in this kit. HBsAg (Adr, CHO expressed) or Ayw (E. coli expressed) were minimally detected in this kit.

## LIMITS OF THE ASSAY

- The recombinant HBsAg antigen in Recombivax HB and Engerix B is absorbed essentially entirely on the aluminum hydroxide adjuvant of each vaccine preparation. In this bound form, the HBsAg is not available for detection in this ELISA assay. The antibodies used in this assay, however, have been shown to quantify the vaccine antigen using another immunoassay format designed for use with bound HBsAg samples.
- Complete **recovery** of HBsAg spiked into human serum and plasma has been shown to occur when the serum/plasma samples are diluted at least 20-fold in the sample diluent contained in the kit.
- HBsAg that is incomplete in sequence (truncated) or is aggregated and/or associated with other biomolecules may not produce dilution curves **parallel** with the Standard curve. For cases of non-parallelism, it may be useful to establish an alternative Standard curve using the altered HBsAg preparation.

## QUALITY CONTROL

**Reagents** Accurate and reproducible assay results rely on proper storage, handling and control of reagent and sample temperature. Store all reagents as indicated, and warm to room temperature only those to be used in the assay. Shelf-life of the critical reagents and samples will diminish with extended exposure to non-refrigeration, resulting in inaccurate assay results. All solutions should be clear. Cloudiness or particulates are indications of reagent contamination or instability and may interfere with proper performance of the assay. Do not use.

**Sample Controls** A Positive Serum Control is provided with the kit, assigned with an HBsAg concentration value range. Recovery in this range is an indicator of proper assay performance. Each lab should also assay internal control samples, which represent the lab's expected sample population and that are maintained stabilized. A Sample Diluent blank should also be run; OD should be <0.3 and lower than 1 ng/ml Standard OD.

**Standard Curve** The signal generated by the standards should be continuously increasing in OD from the lowest Standard to the highest Standard, with a difference greater than 1.2 OD. Non-uniform or low signals may indicate problems with technique, protocol directions and/or reagent preparation, use or stability. Do not rely on results generated from an assay with these issues.

**Technique** Accurate and reproducible assay results rely on good lab technique regarding pipetting, plate washing and handling of samples and reagents.

**Equipment** Precision of results relies on uniform and effective washing techniques; an automatic washer may be used. ELISA reader and pipettes should be properly calibrated.

## STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8°C until the expiration date printed on the box label. Stabilities of the working solutions are indicated under Reagent Preparation.

## RELATED ITEMS

| Catalog#   | Product Description                                   |
|------------|---|
| 4105       | HBsAg ELISA kit, qualitative (diagnostic use)         |
| 4110       | HBsAg (native or recombinant) ELISA Kit, quantitative |
| 4210       | Mouse Anti- HBsAg IgG ELISA Kit                       |
| 4215       | Mouse Anti- HBsAg IgM ELISA Kit                       |
| 4220-AHB   | Human Anti- HBsAg ELISA Kit                           |
| 4230-AHB-R | Human Anti- HBsAg rapid test strips                   |
| 4240       | Rabbit Anti- HBsAg ELISA kit                          |

VAC-HBS-100 VacGel™ | Direct ELISA for the detection and measurement of Hepatitis B vaccine (HBsAg) adsorbed onto Alhydrogel

Instruction Manual No. M-4110

# Hepatitis Surface Antigen (HBsAg) ELISA Kit

Cat. No. 4110, 96 Tests

For Quantitation of Hepatitis Surface Antigen in biological buffers



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