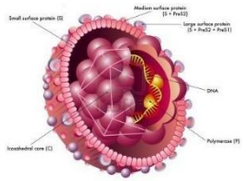
INTENDED USE

The **Human Anti-HBsAg Pre S1 IgG** ELISA Kit detects and quantifies IgG directed to the Pre S1 region **of** hepatitis B surface antigen, in human serum or plasma of vaccinated, immunized and/or infected hosts. Other biological samples may also be tested (e.g., saliva, mucous) with appropriate validation. This immunoassay is suitable for:

- Determining immune status relative to non-immune controls;
- Assessing efficacy of vaccines, including dosage, adjuvantcy, route of immunization and timing;
- Qualifying and/or standardizing vaccine batches and protocols.

For research use only (RUO), not for diagnostic use.

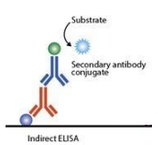
GENERAL INFORMATION



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.

The hepatitis B surface antigen (**HBsAg**) contains the large (L), middle (M), and small (S) surface proteins. The L protein consists of the S protein, **preS1**, and preS2. In HBsAg, the preS domain (preS1 + preS2) plays a key role in the infection of hepatocytic cells by HBV and has several immunogenic epitopes. Based on these characteristics of preS, several preS-based diagnostic and therapeutic materials and systems have been developed. PreS1-specific monoclonal antibodies (e.g., MA18/7 and KR127) can be used to inhibit HBV infection. A myristoylated preS1 peptide (amino acids 2-48) also inhibits the attachment of HBV to HepaRG cells, primary human hepatocytes, and primary tupaia hepatocytes. Antibodies and antigens related to the components of HBsAg, preS (preS1 + preS2), or preS1 can be available as diagnostic markers of acute and chronic HBV infections and to assess the efficacy of experimental vaccines.

PRINCIPLE OF THE TEST



The Human Anti-HB Pre S1 IgG ELISA kit is based on the binding of antibody in samples to HB Pre S1 immobilized on the microwells, and bound antibody is detected by anti-Ig's-specific antibody conjugated to HRP. After a washing step, TMB is added and color (blue) is developed which is directly proportional to the amount of antibody present in the sample. Stopping Solution is added to terminate the reaction, and A450nm is then measured using an ELISA reader. The activity of antibody in samples is determined relative to Calibrators.

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
Wash Solution Concentrate (100x) Cat. No. WB-100, 10ml	Dilute the entire volume 10ml + 990ml with distilled or deionized water into a clean stock bottle. Label as **Wash Solution** and store at ambient temperature until kit is used entirely.
Sample Diluent Concentrate (20x) Cat. No. SD-20T, 10ml	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as **Working Sample/Conjugate Diluent** and store at 2-8°C until the kit lot expires or is used up.
Anti-Human IgG - HRP Conjugate Concentrate (100x) Part No. H-HuG.2a11, 0.15ml	Peroxidase conjugated anti-Human IgG in buffer with protein, detergents and antimicrobial as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of **Working Sample/Conjugate Diluent** 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
HB Pre S1 Antigen Microwell Strip Plate	4301	8-well strips (12)	Coated with recombinant HB Pre S1 , and post-coated with stabilizers.
Anti-HB Pre S1 Calibrators			
1 U/ml 2.5 U/ml 5 U/ml 10 U/ml	4302B 4302C 4302D 4302E	0.65 ml 0.65 ml 0.65 ml 0.65 ml	Four (4) vials, each containing anti-HB Pre S1 in arbitrary activity Units; diluted in buffer with protein, detergents and antimicrobial as stabilizers.
Anti-HB Pre S1 Positive Control	4302PC	0.65ml	Anti-HB Pre S1 diluted in buffer with protein, detergents and antimicrobial as stabilizers. [Value Range on Label]
Low NSB Sample Diluent	TBTm **Not** for conjugate dilution	30 ml	Buffer with protein, detergents and antimicrobial as stabilizers. Use as is for sample dilution
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	Dilute 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 Anti-Human IgG HRP Concentrate.
- Graduated cylinder to dilute Wash Concentrate; 200ml 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

Sample Collection and Handling

Serum and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference. For **serum**, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. If samples will not be assayed immediately, store refrigerated for up to a few weeks, or frozen for long-term storage.

Caution: Human serum and other bodily fluids may contain infectious material. Always wear gloves when handling human samples, including the standards and controls (which have been tested non-reactive for HB Pre S1 and Anti-HIV), and dispose of these samples and containers as biohazard waste.

Antibody Stability & Dilution

Initial dilution of serum into **Working Sample Diluent** (WSD) is recommended to stabilize antibody activity. This enhances reproducible sampling, and stabilizes the antibody activity for years, stored refrigerated or frozen. Further dilution into **Low NSB Sample Diluent** (LNSD), which provides the lowest assay background, should be at least 10 times the initial dilution and performed the same day as the assay.
Example: Initial (1:5): **10**ul serum + **40**ul WSD [or 0.1ml + 0.4ml]
Further (1:50): **10**ul initial (1:5) + **90**ul LNSD (1:50)

Assay Design

Review Interpretation of Results (p5-7) before proceeding:

- Select the proper sample dilutions accounting for expected potency of positives and minimizing non-specific binding (NSB) and other matrix effects; for example, net signal for non-immune samples should be lower than the **1 U/ml Calibrator**. This is usually 1:100 or greater dilution for human serum with normal levels of IgG and IgM.
- Run the **Anti-HB Pre S1 Positive Control**; value range is on the label.
- Run a Sample Diluent **Blank**. This signal is an indicator of proper assay performance, especially of washing efficacy, and is used for net OD calculations, if required. Blank OD should be <0.3.
- Run a set of **Calibrators**, which validate that the assay was performed to specifications: **10 U/ml** should give a high signal (>1.5 OD); **1 U/ml** should give a low signal which can be used to discriminate at the Positive/Negative threshold (see Interpretation of Results, p. 5).

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 8 Calibrator wells and 2 wells for each sample 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.

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 8 Calibrator 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. 1st 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. 2nd Incubation [100ul – 30 min; 5 washes]

- Add 100ul of diluted Anti-Human IgG HRP 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.

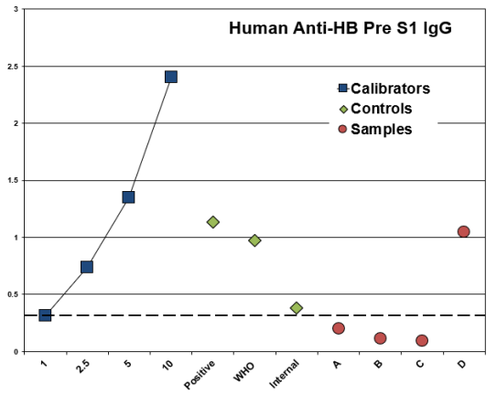
INTERPRETATION OF RESULTS

A. Antibody Activity Threshold Index

Compare Samples to **10 U/ml Calibrator** or **Internal Control**

= Positive/Negative Cut-off.

Example:



Results

The **sensitivity** of the assay to detect anti-HB Pre S1 IgG, from either natural infection or vaccination, is controlled so that the **1 U/ml Calibrator** represents a threshold OD for most true positives in human serum diluted to 1:100 or greater. Visual inspection of the data in the above graph shows the following:

Calibrators – dilution curve of antiserum from anti-HB Pre S1 immunization, shows the OD range of the assay; high value indicates optimal sensitivity of the assay.
1 U/ml: a 'Cut-off' line has been drawn to indicate a threshold distinguishing between **Positive/Negative**. This is not a clear-cut threshold, rather a low OD area that could represent either low positives or high background negatives.

Positive Control – an anti-HB Pre S1 serum; value range is on the label. This Control can be used to gauge precision and to normalize between-assay variation.

WHO International Standard for **Anti-HBsAg** [NIBSC 07/164]: Diluted to **1** IU/ml = **3.2** U/ml Pre S1 in the assay.

Internal Control – a true positive from an immune human that represents the investigator's experience in distinguishing low positive from negative samples (not in kit). This should be run in each assay to supplement the 10 U/ml Calibrator for Positive/Negative discrimination purposes.

Samples A,B,C,D – 3 samples (1:100) (A, B, C) are negative: below the threshold; 1 sample (D) is positive: clearly above the threshold.

The 1 U/ml Calibrator can be used to calculate a **Threshold Index** that numerically discriminates Positive/Negative:

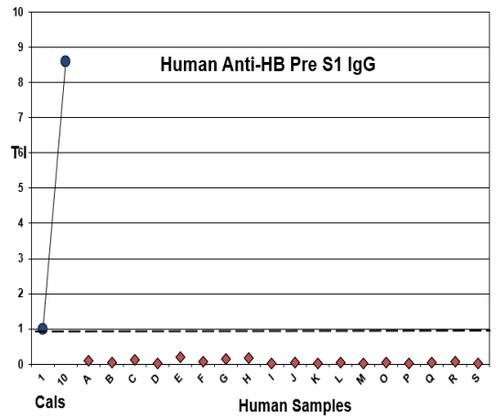
- Divide each Sample net OD by the 1 U/ml Calibrator net OD. Values above 1.0 are a measure of **Positive** Antibody Activity; below 1.0 are **Negative** for antibody.

INTERPRETATION OF RESULTS (cont)

Example:

Human Serum IgG

A panel of human serum/plasma of unknown history was tested for anti-HB Pre S1 IgG (1:200 dilution). **Threshold Index** was calculated using the **1** U/ml **Cal**.



Results

Anti- HB Pre S1 IgG: all eighteen (18) samples were negative (below the **1.0** Theshold Index) 1:200 dilution.

Notes:

1. **Positives** may be due to prior encounter with the virus, similar antigen(s), or anti-HBV immunization.
2. The **sensitivity** of the assay may be adjusted by changing the sample dilutions: a) increase dilution (e.g., 1:500) to lower the signals of borderline positives to negative; b) decrease dilution (e.g., 1:100) to convert borderline samples to positive. With the latter, the values of negatives may increase, so an alternative threshold should be considered using known negatives to develop a **Positive Index (**see below) or use an **Internal Control** (Page 5).
3. If other biological specimens and/or HB Pre S1 preparations, are used for antibody determination, sample dilutions and positive/negative thresholds should be determined using specimens from non-immune or pre-immune populations.

B. Positive Index
Experimental sample values may be expressed relative to the values of Control or Non-immune samples, by calculation of a **Positive Index**. One typical method is as follows:

1. Calculate the net OD mean + 2 SD of the Control/Non-immune samples = **Positive Index**.
2. Divide each sample net OD by the Positive Index. Values above 1.0 are a measure of **Positive** Antibody Activity; below 1.0 are **Negative** for antibody.

INTERPRETATION OF RESULTS (cont)

A sample value would be **Positive** if significantly above the value of the pre-immune serum sample or a suitably determined non-immune panel or pool of samples, tested at the same sample dilution.

This calculation also **quantifies** the positive Antibody Activity level, assigning a higher value to samples with higher Antibody Activity, and vice versa.

Method C. Titers from Sample Dilution Curves
The titer of elevated antibody activity calculated from a dilution curve of each sample is recommended as the most accurate quantitative method. Best precision can be obtained using the following guidelines:

1. Use an OD value Index in the mid-range of the assay (2.0 – 0.5 OD); this provides the best sensitivity and reproducibility for comparing experimental groups and replicates. An arbitrary 1.0 OD is commonly used.
2. Prepare serial dilutions of each sample to provide a series that will produce signals higher and lower than the selected index. With accurate diluting, duplicates may not be required if at least 4 dilutions are run per sample.
3. A 5-fold dilution scheme is useful to efficiently cover a wide range which produces ODs both above and below 1.0 OD. The dilution scheme can be tightened to 3-fold or 2-fold for more precise comparative data.
4. The Positive and Sensitivity Control values can be used to normalize inter-assay values.

Calculations

1. On a log scale of inverse of Sample Dilution as the x-axis, plot the OD values of the two dilutions of each positive sample having ODs above and below the OD value of the Index (arbitrary or selected Calibrator).
2. From a point-to-point line drawn between the two sample ODs, read the dilution value (x-axis) corresponding to the OD of the selected Index
   = IgG Antibody Activity Units

PRODUCT SPECIFICATIONS

Specificity
Purified HB Pre S1 peptide (5.2 kDa) is used to coat the microwells; stabilizing postcoat contains BSA; thus, no other antibody specificity is detectable in the assay. The anti-human IgG HRP conjugate specifically detects IgG, and will not react with IgM, IgA or IgE class antibodies.

Assay Sensitivity
The HB Pre S1 -coated plated, the anti-human IgG HRP concentration, and the Low NSB Sample Diluent are optimized to differentiate anti-HB Pre S1 IgG from background (non-antibody) signal with human serum/plasma samples diluted 1:100.

PRECAUTIONS AND SAFETY INSTRUCTIONS

Calibrators, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains dilute 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&category_id=2430&visit=10

Instruction Manual No. M-4300

Human Anti-HBsAg Pre S1 IgG

ELISA Kit Cat. No. 4300

For Quantitation of Anti-Hepatitis B Surface Antigen Pre S1 [HB Pre S1] IgG In Serum or Plasma

For research use only, not for diagnostic or therapeutic use.

ALPHA DIAGNOSTIC INTERNATIONAL

4638 N Loop 1604 W • San Antonio• Texas 78249 • USA.

Phone (210) 561-9515 • Fax (210) 561-9544

Toll Free (800) 786-5777

Email: service@4adi.com

ELISA Kit Components	Amount	Part
HB Pre S1 Coated Microwell Strip Plate	8-well strips (12)	4301
Anti-HB Pre S1 Positive Control	0.65 ml	4302PC
Anti-HB Pre S1 Calibrator 1 U/ml	0.65 ml	4302B
Anti-HB Pre S1 Calibrator 2.5 U/ml	0.65 ml	4302C
Anti-HB Pre S1 Calibrator 5 U/ml	0.65 ml	4302D
Anti-HB Pre S1 Calibrator 10 U/ml	0.65 ml	4302E
Anti-Human IgG HRP Conjugate (100X)	0.15 ml	H-HuG.2a11
Sample Diluent (20X)	10 ml	SD20T
Low NSB Sample Diluent	30 ml	TBTm
Wash Solution Concentrate (100X)	10 ml	WB-100
TMB Substrate	12 ml	80091
Stop Solution	12 ml	80101
Product Manual	1 ea	M-4300