ELISA kits available from ADI (see details at the web site)

#0010 Human Leptin
#200-120-AGH Human globular Adiponectin (gAcrp30)
#0700 Human Sex Hormone Binding Glob (SHBG)
#0900 Human IGF-Binding Protein 1 (IGFBP1)
#1000 Human C-Reactive Protein (CRP)
#100-110-RSH Human Resistin /FIZZ3
#100-140-ADH Human Adiponectin (Acrp30)
#100-160-ANH Human Angiogenin
#100-180-APH Human Angiopoietin-2 (Ang-2)
#100-190-B7H Human Bone Morphogenetic Protein 7 (BMP-7)
#1190 Human Serum Albumin #1200 Human Albumin (Urinary)
#1750 Human IgG (total) #1760 Human IgM
#1800 Human IgE #1810 Human Ferritin
#1210 Human Transferrin (Tf) #0020 Beta-2 microglobulin
#1600 Human Growth Hormone (GH)

#0060 Human Pancreatic Colorectal cancer (CA-242)
#1820 Human Ovarian Cancer (CA125) #1830 Human CA153
#1840 Human Pancreatic & GI Cancer (CA199)
#1310 Human Pancreatic Lipase
#1400 Human Prostatic Acid Phosphatase (PAP)
#1500 Human Prostate Specific Antigen (PSA) #1510 free PSA (fPSA)
#1500 Human Alpha Fetoprotein (AFP)
#0050 Human Neuron Specific Enolase (NSE)

#0030 Human Insulin #0040 Human C-peptide
#0100 Human Luteinizing Hormone (LH)
#0200 Human Follicle Stimulating Hormone (FSH)
#0300 Human Prolactin (PRL)
#0400 Human Chorionic Gonadotropin (HCG) #0410 HCG-free beta

#0600 Human Thyroid Stimulating Hormone (TSH)
#1100 Human Total Thyroxine (T4) #1110 Human Free T4 (fT4)
#1650 Human free triiodothyronine (FT3) #1700 Human T3 (total)

#1850 Human Cortisol #1860 Human Progesterone
#1865 Human Pregnanolone #1875 Human Aldosterone
#1880 Human Testosterone #1885 Human free Testosterone
#1910 Human Androstenedione #1920 Human Estradiol
#1925 Human Estrone #1940 Dihydrotestosterone (DHT)
#1950 Human DHEA-sulphate (DHEA-S)
#3400 Human serum Neopterin

#3000 Human Rheumatoid Factors IgM (RF)
#3100 Human anti-dsDNA
#3200 Anti-Nuclear Antibodies (ANA)

Hepatitis B Surface Antigen (HBsAg)

ELISA KIT Cat. No. 4100

For Quantitative Determination of HBsAg In Human Serum or Other Fluids

For In Vitro Research Use Only

6203 Woodlake Center Drive • San Antonio • Texas 78244 • USA.
Phone (210) 561-9515 • Fax (210) 561-9544
Toll Free (800) 786-5777
Email: service@4adi.com
Web Site: www.4adi.com
**Kit Contents:** (reagents for 96 tests)

<table>
<thead>
<tr>
<th>Components</th>
<th>96 tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-HBsAg IgG microwell strip plate (96 wells), #4101</td>
<td>1 Plate</td>
</tr>
<tr>
<td>HBsAg standard A, 0 ng/mL, 0.7 ml, #4102A</td>
<td>1 vial</td>
</tr>
<tr>
<td>HBsAg standard B, 0.5 ng/mL, 0.7 ml, #4102B</td>
<td>1 vial</td>
</tr>
<tr>
<td>HBsAg standard C, 1 ng/mL, 0.7 ml, #4102C</td>
<td>1 vial</td>
</tr>
<tr>
<td>HBsAg standard D, 2.5 ng/mL, 0.7 ml, #4102D</td>
<td>1 vial</td>
</tr>
<tr>
<td>HBsAg standard E, 5 ng/mL, 0.7 ml, #4102E</td>
<td>1 vial</td>
</tr>
<tr>
<td>HBsAg standard F, 10 ng/mL, 0.7 ml, #4102F</td>
<td>1 vial</td>
</tr>
<tr>
<td>Anti-HBsAg IgG-HRP Conjugate, 6 ml, # 4103</td>
<td>1 bottle</td>
</tr>
<tr>
<td>Wash Buffer (100X); 10 ml, dilute 1:100 with distilled water; Cat# W-100</td>
<td>1 bottle</td>
</tr>
<tr>
<td>TMB Substrate Solution # 4100-TMB, 11 ml</td>
<td>1 bottle</td>
</tr>
<tr>
<td>Stop solution, 10 ml, Cat. # T-10</td>
<td>1 bottle</td>
</tr>
<tr>
<td>Instruction Manual, M - 4 1 0 0</td>
<td>1</td>
</tr>
</tbody>
</table>

**INTRODUCTION AND EXPLANATION**

The discovery of Australian antigen by Blumberg et al., and its subsequent identification as the surface antigen of hepatitis B virus (HBsAg) represents a significant breakthrough in the understanding of the disease, serum hepatitis. Screening of blood donors for the presence of this antigen in serum has significantly reduced the incidence of hepatitis B in blood transfusion recipients.

The chemical composition of the HBsAg consists of lipid, carbohydrate and protein. The protein moiety of HBsAg contains several polypeptides, ranging from 23,000 to 97,000 molecular weight. The antigenic determinants on the protein moiety of the HBsAg determine the specific characteristics of the different serotypes of the virus and are the basis of the immunoassay. The antigenic reactivity of HBsAg is also associated with the surface of spherical or tubular particles. Other particles have also been observed, called Dane particles, which have two different antigenic sites: a superficial one, identifiable an HBsAg and an inner one, identifiable as the core. It has also been suggested that HBsAg is a fragment of the viral lipoprotein capsid and the Dane particle could be the real virus. HBsAg has an antigenic heterogeneity. The principal determinant is called a (al, a2, a3) and is common to all the different serotypes of HBsAg. Two copies of subspecific determinants have also been identified, that is d/y (1l, 2y, 3y) and w/r which seems to be mutual. Therefore the following combinations are possible: adw, adr, ayw, ayr.

**Interpretation of Results**

Compare the color of the patient samples well to the color of the positive and the negative reference wells.

**Negative**

Samples that developed no color or less color than the –ve control or cut-off value (A450 of zero standard+0.1=cut off values).

**Positive**

Samples that developed the color equal to or stronger than the positive reference (cut-off value) wells are considered positive.

**Accuracy**

This ELISA kit meets the requirements for a 3rd generation test when tested against the FDA reference panel.

**Sensitivity**

The serial dilutions of HBsAg were prepared in recalcified human plasma. A linear study was performed to determine the sensitivity. Based upon cut-off value=0.1+mean negative control, the sensitivity is determined to 0.3 ng/ml

**Comparison Study**

The comparison studies are carried out on 300 random patient samples from a clinical laboratory with a commercial test kit (Abbot Auszyme II) and ADI’s ELISA test kit.

<table>
<thead>
<tr>
<th>Results</th>
<th>ADI’s ELISA Kit</th>
<th>Abbot Auszyme II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>75</td>
<td>74</td>
</tr>
<tr>
<td>Negative</td>
<td>225</td>
<td>226</td>
</tr>
<tr>
<td>Total</td>
<td>300</td>
<td>300</td>
</tr>
</tbody>
</table>

**Specificity**

<table>
<thead>
<tr>
<th>Source of Specimen</th>
<th>Tested</th>
<th>Positive</th>
<th>negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver disease</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Patient associated with Cytomegalovirus</td>
<td>Patient with: Cytomegalovirus</td>
<td>Patient with: Cytomegalovirus</td>
<td></td>
</tr>
<tr>
<td>EB virus</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Herpes Virus</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Viral hepatitis A</td>
<td>20</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Non-A/Non-B hepatitis</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Alcoholism</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>0</td>
<td>32</td>
</tr>
</tbody>
</table>
**WORKSHEET OF TYPICAL ASSAY**

<table>
<thead>
<tr>
<th>Wells</th>
<th>Stds/samples</th>
<th>Mean $A_{450nm}$</th>
<th>Calculated Conc. (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1, A2</td>
<td>(0 ng/mL)</td>
<td>0.025</td>
<td></td>
</tr>
<tr>
<td>B1, B2</td>
<td>(0.5 ng/mL)</td>
<td>0.216</td>
<td></td>
</tr>
<tr>
<td>C1, C2</td>
<td>(1 ng/mL)</td>
<td>0.382</td>
<td></td>
</tr>
<tr>
<td>D1, D2</td>
<td>(2.5 ng/mL)</td>
<td>0.928</td>
<td></td>
</tr>
<tr>
<td>E1, E2</td>
<td>(5 ng/mL)</td>
<td>1.719</td>
<td></td>
</tr>
<tr>
<td>F1, F2</td>
<td>(10 ng/mL)</td>
<td>3.014</td>
<td></td>
</tr>
<tr>
<td>G1, G2</td>
<td>Negative Sample 1</td>
<td>0.323</td>
<td>0.796</td>
</tr>
</tbody>
</table>

**NOTE:** These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.

A typical std. assay curve for HBsAg (do not use this for calculating sample values)

**PRINCIPLE OF THE TEST**

The assay is a direct Sandwich\(^*\) test based on the use of microtiter wells on which monoclonal mouse IgG to HBsAg are coated and other polyclonal antibody is in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the two antibodies resulting in the HBsAg molecule being sandwiched between the solid phase and enzyme linked antibodies. Unbound enzyme conjugate is then removed and the wells washed. The chromogen substrate solution containing hydrogen peroxide is added to the well and, after incubation, a blue color develops in proportion to the amount of HBsAg which is bound to the well. The enzyme reaction is stopped by the addition of acid. Within limits the greater the amount of antigen in the specimen, the higher the absorbance. Specimens giving absorbance equal to or greater than the cutoff value (the absorbance value of negative control mean plus a factor of 0.06) are considered reactive for HBsAg. Specimens, which are repeatedly reactive by this assay, are considered positive for the presence of HBsAg. Specimens with absorbance value less than the cutoff value is considered negative for HBsAg.

**MATERIALS AND EQUIPMENT REQUIRED**

Adjustable micropipet (25-100 \(\mu l\)) and Multichannel pipet with disposable plastic tips. Reagent troughs, Plate washer (recommended) and ELISA plate Reader.

**PRECAUTIONS**

The Alpha Diagnostic Intl., Inc. HBsAg ELISA test is intended for in vitro research use only. The reagents contain proclin-300 as preservative; necessary care should be taken when disposing solutions. The Control Serum has been prepared from human sera shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses, therefore, sera should be handled with appropriate precautions.

Applicable MSDS, if not already on file, for the following reagents can be obtained from ADI or the web site. TMB (substrate), H2SO4 (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).

All waste material should be properly disinfected before disposal. Avoid contact with the stop solution (1N sulfuric acid).

**SPECIMEN COLLECTION AND HANDLING**

The ADI's HBsAg ELISA test is performed on human serum, plasma or reclassified plasma. HBsAg is thermolabile. If specimens are not to be tested they should be refrigerated immediately at 2\(^\circ\)-8\(^\circ\)C. Also, if storage periods greater than 3 days are anticipated, the specimen should be frozen. If specimens are to be shipped, they should be packed in compliance with applicable regulations covering the transportation of etiologic agents. Specimens containing precipitate may give inconsistent results and should be clarified prior to assaying.

**Reagent Preparation:**

Dilute wash buffer (1:100) with distilled water (10 ml stock in total of 1-liter)
STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8°C until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping. Standards are stable for two month at 2-8°C. The unused portions of the standards can be frozen in suitable aliquots for long-term use. Repeated freezing and thawing is not recommended.

TEST PROCEDURE (ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE). Prepare 1X wash buffer by diluting it with water (1:100 or mix the entire content of the bottle ion 1 liter of water). Store at room temp for the day or keep at 4°C. Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag.

1. Label or mark the microtiter well strips to be used on the plate.

2. Pipet 50 ul of standards, controls, and serum samples into appropriate wells in duplicate.

3. Add 50 ul of enzyme conjugate into each well. Mix gently for 5 seconds. Cover the plate and incubate for 60 minutes at 37°C (incubating at room temp will lower the A450 values but it can be optimized).

4. Aspirate and wash the wells 5 times with 300 ul of 1X wash buffer. We recommend using an automated ELISA plate Washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.

5. Dispense 100 ul TMB solution (HRP substrate) into each well and Mix gently for 5-10 seconds. Cover the plate and incubate for 30 minutes at room temperature. Blue color develops into standards and positive samples. This incubation step can be varied 5 ± minutes to achieve optimal color ~A450=2.00.

6. Stop the reaction by adding 50 ul of stop solution to all wells at the same timed intervals as in step 6. Mix gently for 5-10 seconds. Blue color turns yellow.

7. Measure the absorbance at 450 nm using an ELISA reader within 30 min.

NOTES

Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 4°C.

Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

DILUTION OF SAMPLES

Serum samples do not usually require dilution. However, if dilution is desired, the zero standard (Sample Diluent) must be used and the results obtained should be multiplied by the appropriate dilution factor.

QUALITY CONTROL

Results of an assay run are valid if the following criteria are met: The mean absorbance of Negative Control should be less than 0.15.. The absorbance of the positive control should be >0.35.

CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Subtract the absorbance of the zero standard from the mean absorbance values of standards, control, and samples. Draw the standard curve on log-log graph paper by plotting net absorbance values of standards against appropriate HBsAg concentrations. Read off the HBsAg concentrations of the control and patient samples.