ELISA kits available from ADI:

<table>
<thead>
<tr>
<th>Catalog#</th>
<th>ProdDescription</th>
</tr>
</thead>
<tbody>
<tr>
<td>920-500-MBG</td>
<td>Mouse Anti-Influenza B IgG ELISA kit, Quantitative</td>
</tr>
<tr>
<td>920-600-RBG</td>
<td>Rabbit Anti-Influenza B IgG ELISA kit, Quantitative</td>
</tr>
<tr>
<td>930-100-TTH</td>
<td>Human Anti-Tetanus Toxoid IgG ELISA kit</td>
</tr>
<tr>
<td>930-110-TTM</td>
<td>Mouse Anti-Tetanus Toxoid IgG ELISA kit</td>
</tr>
<tr>
<td>940-120-DMG</td>
<td>Mouse Anti-Diphtheria IgG ELISA kit, Quantitative</td>
</tr>
<tr>
<td>960-150-PRG</td>
<td>Rabbit Anti-B. Pertussis IgG ELISA kit, Quantitative</td>
</tr>
<tr>
<td>4100</td>
<td>Hepatitis B Surface Antigen (HBsAg) ELISA kit, Quantitative</td>
</tr>
<tr>
<td>4210</td>
<td>Mouse Anti-Hepatitis B Surface Antigen (anti-HBsAg) ELISA kit, Quantitative</td>
</tr>
<tr>
<td>4215</td>
<td>Mouse Anti-Hepatitis B Surface Antigen (anti-HBsAg) IgM ELISA kit, Quantitative</td>
</tr>
<tr>
<td>4220-AHB</td>
<td>Anti-Hepatitis B Surface Antigen (anti-HBsAg) ELISA kit, Quantitative</td>
</tr>
<tr>
<td>4230-AHB-R</td>
<td>Anti-Hepatitis B Surface Antigen (anti-HBsAg) rapid test strips</td>
</tr>
<tr>
<td>4300-AHG</td>
<td>Anti-Hepatitis A Virus IgG (HAV-IgG) ELISA kit, Quantitative</td>
</tr>
</tbody>
</table>


Monkey: IgM, IgG, IgA, IgE

Rat: Albumin, CRP, IgG, IgM, Alpha-1- Acid glycoprotein

Mouse: Albumin, IgA, IgG, IgG1, IgG2a, IgG2b, IgG3, IgE, IgM, Leptin, Resistin, Acrp30, CRP, Troponin-I, TNF-alpha

Autoimmune Antibody detection kits for ANA, ssDNA, dsDNA, Histone, Sm, RNP, SSA, SSB, Sci70, Ovalbumin, Cardiolipin, and CIC

Chicken: IgG, IgM, IgY, Ovalbumin

Turkey: IgG

Bovine: Albumin, IgG, IgM, Lactoferrin, Transferrin

Pig: Albumin, IgG, IgM

Dog: CRP, IgG, IgM

Cat: IgG, IgM

Sheep: IgG

Goat: IgG

Rabbit: CRP, IgG

See Details at the web site or Contact ADI

Mouse Anti-B. Pertussis IgG

ELISA KIT Cat. # 960-130-PMG

For Quantitative Determination of Mouse B. Pertussis IgG in Serum/Plasma

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
**CALCULATION OF RESULTS**

**Qualitative Method (-ve and +ve determinations)**

This kit is supplied with normal mouse negative control. It is only meant to represent a range of values expected in normal, non-immunized or non-vaccinated animals. The basal antibody level may differ in normal animals due to age, strain, and exposure to the hepatitis virus or the antigens. Therefore, we recommend that you determine the “NORMAL VALUES” of your own control population and not compare it with the control provided in the kit.

**Negatives** - All values that are equal or less than the –ve control A450 values (e.g., 0.083) when tested at recommended dilution (1:100) can be considered negative.

**Positive** - All values that are equal or higher than the calculated cut-off A450 values (0.69+0.200=0.89) when tested at recommended dilution can be considered positive. An arbitrary value of 0.200 is added to the actual value of 0.69 to calculate the cut-off values.

**Quantitative Method**

1. Calculate the mean absorbance for each duplicate. Subtract the values of the blanks (sample diluent) form all values (standards and controls) to get the net A450 values. Draw the standard curve graph paper by plotting calculated net A450 values of standards against appropriate concentrations. Read off the concentrations of the control and samples.

2. If samples were diluted 1:100 then it is not necessary to multiply the values by 100 (this dilution has been taken into account for the standards). If samples were diluted by 1:200 then multiply the calculated values by 2. Samples diluted 1:400 should be multiplied by 400/100 = 4.

If available, graphing software may be used to analyze the data. Depending on the range of the standard curve used, we find that good fits of the data may be obtained using a point-to-point fit.

**PERFORMANCE CHARACTERISTICS**

**Detection Limit**: The minimum concentration of B. Pertussis IgG detectable using this assay is 20 U/ml.

**Specificity**: The antibodies used in this kit are specific for Mouse B. Pertussis and have shown no cross-reactivity with other B. Pertussis or proteins. The conjugate used detects only the IgG isotype (anti-B. Pertussis). The conjugate used detects only the IgG isotype (anti-diphtheria). ADI has other kits that measure IgM isotype (anti-diphtheria).

**Species Crossreactivity**: Cross-reactivity of Mouse B. Pertussis IgG ELISA kit with other animals has not been tested. ADI has other kits to detect anti-Diphtheria Toxoid IgG in human, mouse, and rabbit samples.
### WORKSHEET OF TYPICAL ASSAY

<table>
<thead>
<tr>
<th>Wells</th>
<th>Stds/samples</th>
<th>Mean A450</th>
<th>Calculated values (A450)</th>
<th>Calculated conc. U/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1, A2</td>
<td>Diluent only blanks</td>
<td>0.010</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B1, B2</td>
<td>Standard A, 1.0 U/ml</td>
<td>0.036</td>
<td>0.026</td>
<td></td>
</tr>
<tr>
<td>C1, C2</td>
<td>Standard B, 20 U/ml</td>
<td>0.553</td>
<td>0.543</td>
<td></td>
</tr>
<tr>
<td>D1, D2</td>
<td>Standard C, 45 U/ml</td>
<td>1.512</td>
<td>1.502</td>
<td></td>
</tr>
<tr>
<td>E1, E2</td>
<td>Standard D, 150 U/ml</td>
<td>2.201</td>
<td>2.191</td>
<td></td>
</tr>
<tr>
<td>F1, F2</td>
<td>Sample 1 (1:100)</td>
<td>1.633</td>
<td>1.622</td>
<td>53</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 assay Curve (do not use this for calculating sample values)

### PRINCIPLE OF THE TEST

Anti-B. Pertussis B IgG ELISA kit is based on binding of Anti-B. Pertussis IgG to the antigen immobilized on microtiter wells. After a washing step, goat anti-mouse Ig-HRP conjugate is added. After another washing step, to remove all the unbound enzyme conjugate, chromogenic substrate (TMB) is added and color developed. The enzymatic reaction (blue color) is directly proportional to the amount of Anti-B. Pertussis IgG present in the sample. The reaction is terminated by adding stopping solution (converts blue to yellow). Absorbance is then measured on a microtiter well ELISA reader at 450 nm.

### MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5-1000 ul) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plates Reader.

### PRECAUTIONS AND SAFETY INSTRUCTIONS

The Mouse B. Pertussis IgG ELISA Kit is for research use only.

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, if not already on file, can be requested or obtained from the ADI website.

### SPECIMEN COLLECTION and HANDLING

Collect blood by venipuncture; allow clotting, and separating the serum by centrifugation at room temperature. Do not heat inactivate the serum. Plasma can also be used. If sera cannot be immediately assayed, store frozen for up to six months. Avoid repeated freezing and thawing of samples. Cell or tissues extract samples have not been optimized.

### STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8oC until the expiration date printed on the label.
REAGENT PREPARATION

**Wash Buffer Concentrate** (*10X solution*). Before use, dilute the 10 ml of concentrate with 90 ml of distilled or deionized water. Occasionally, some salts may form crystals during storage in cold but they redissolve upon slight warming of the solution.

DILUTION OF SAMPLES

We recommend a serum samples to be diluted 1:100 or more to suppress the background. All samples should be diluted in 1X sample diluent. We are recommending 2 step scheme to save the diluent. It is possible to perform sample dilution in a single step.

Sample dilution can be performed in 1 step;
5 ul sample and 495 ul 1x diluent (dilution 1:100)

TEST PROCEDURE

*(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE)*

1. The calibrators are supplied as ready-to-use, and their concentrations are 1, 20, 45 and 150 U/ml. Immediately aliquot and store any unused calibrators at –20°C or below.

<table>
<thead>
<tr>
<th>B. Pertussis Stds</th>
<th>Stock Volume</th>
<th>Sample diluent</th>
<th>Final Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Std D (150 U/ml)</td>
<td>250 uL of Cal.</td>
<td>0</td>
<td>250 uL</td>
</tr>
<tr>
<td>Std C (45 U/ml)</td>
<td>250 uL of Cal.</td>
<td>0</td>
<td>250 uL</td>
</tr>
<tr>
<td>Std B (20 U/ml)</td>
<td>250 uL of Cal.</td>
<td>0</td>
<td>250 uL</td>
</tr>
<tr>
<td>Std A (1.0 U/ml)</td>
<td>250 uL of Cal.</td>
<td>0L</td>
<td>250 uL</td>
</tr>
</tbody>
</table>

**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 2-8°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.

Quality Control

Please read the instruction manual and follow the suggested protocol for sample preparations and assay.

**Sample Diluent blanks** – must be <0.200, if higher then repeat the test and increase number of washings.

**Standard A**- Must be <0.200, if higher then repeat the test and increase number of washings.

**Standards D or Positive Control**- Must be >1.20, if less then repeat the test. All controls must fall within the specified limits for the assay to be valid.