Procedure: All reagents must be at room temperature prior to use.

1. Take out the coated plate or required number of 8-well strips and record the sample position on a worksheet.

2. Dilute samples 1:101 with sample diluent (example: 2 ul sample and 200 ul diluent). Need 200 ul per sample or 100 ul x 2 for duplicates. Do not dilute Negative and positive control serum as they are supplied pre-diluted.

3. Dispense 100 ul of controls and samples in duplicate. Set 2 wells for blank diluent wells (Add 100 ul sample diluent). Mix gently for 5-10 seconds and incubate at 37°C for 30 min.

4. Remove or aspirate the well contents and Wash each well 4X with 300 ul. Tap the plate over clean, dry paper towels or absorbent paper to remove traces of well contents.

5. Add 100 ul enzyme conjugate into each well, and incubate at 37°C for 30 min.

6. Wash the plate 4X as in step 4.

7. Pre-mix required amounts of Substratesolution A and B in a separate tube in 1:1 ratio (example: For 2 ml substrate, take 1ml of Soln A and 1 ml of Soln B). Prepare 1 ml for each 8-well strip or 10 ml for full plate by mixing 5 ml of Solns A and 5 ml of Soln B. Add 100 ul or mixed substrate solution into each well, mix gently for 5-10 seconds and incubate for 10 min at room temperature in the dark. Color develops in positive wells.

8. Add stop solution one drop (50 ul) to each well, mix gently for 5-10 seconds and determine the result within 10 min. Read the plate at 450 nm using 630nm as reference values in an ELISA reader.

CALCULATION OF RESULTS

Deduct the average blank values (typically <0.100) from all controls and samples.

Negatives: Net A450 values (minus blanks) =<0.200

Positives: Net A630 values (minus blanks) equal to or >0.400

Unequivocal: Net A450 values =0.200-0.400

Quality Controls

Blanks and Control must be within the specified range. High blanks >0.300 is usually due to insufficient washing.

Specificity of the test

This test detects the IgG-subclass of MDV antibodies, and IgM and IgA are not detected. This kit is recommend for chicken samples and other birds are not tested.
Anti-Marek’s Disease Virus (MDV) ELISA KIT # 920-130-MDV

<table>
<thead>
<tr>
<th>Kit Components, 96 tests</th>
<th>#</th>
</tr>
</thead>
<tbody>
<tr>
<td>MD Antigen coated microtiter plate (96 wells/plate)</td>
<td>920131-P</td>
</tr>
<tr>
<td>Negative serum, white cap (0.8 mL)</td>
<td>920130NC</td>
</tr>
<tr>
<td>Positive serum, red cap (0.8mL)</td>
<td>920130PC</td>
</tr>
<tr>
<td>HRP conjugate (12 mL)*</td>
<td>920132</td>
</tr>
<tr>
<td>concentrated Wash buffer10X (50 mL)</td>
<td>920130-WB</td>
</tr>
<tr>
<td>Substrate A solution, white cap (6 mL)</td>
<td>920130-SA</td>
</tr>
<tr>
<td>Substrate B solution, brown cap (8 mL)</td>
<td>920130-SB</td>
</tr>
<tr>
<td>Stop solution, white cap (6 mL)</td>
<td>920130-ST</td>
</tr>
<tr>
<td>Sample diluent solution, transparent cap (50 mL)</td>
<td>920130-SD</td>
</tr>
<tr>
<td>Instruction Manual</td>
<td>920-130-MDV-M</td>
</tr>
</tbody>
</table>

**Intended Use**

Alpha Diagnostic Intl’s Anti-Marek’s Disease Virus (MDV) ELISA Test kit is a highly sensitive indirect type assay for the detection of MDV antibodies in Chicken serum or plasma. This kit is for research use only.

**Introduction**

Marek’s disease virus (MDV) is a highly contagious viral infection that predominantly affects chickens. It is named after József Marek, a Hungarian veterinarian. The disease is one of the most common diseases affecting poultry flocks worldwide. Mortality rates can be very high in susceptible birds. It is caused by an alphaherpervirus known as ‘Marek’s disease virus’ (MDV) or Gallidherpesvirus 2 (GaHV-2). MDV is an enveloped dsDNA virus (~120-180 Kb) that codes for several capsid proteins (gB, gC, gD, and gH proteins).

The disease is characterized by the presence of T cell lymphoma as well as infiltration of nerves and organs by lymphocytes. Birds infected with GaHV-2 can be carriers and shedders of the virus for life. Newborn chicks are protected by maternal antibodies for a few weeks. After infection, microscopic lesions are present after one to two weeks, and gross lesions are present after three to four weeks. The virus is spread in dander from feather follicles and transmitted by inhalation.

Classical Marek’s disease or neurolymphomatosis causes asymmetric paralysis of one or more limbs. With vagus nerve involvement, difficulty breathing or dilatation of the crop may occur. Besides lesions in the peripheral nerves, there are frequently lymphomatous infiltration/tumours in the skin, skeletal muscle, visceral organs. Organs that are commonly affected include the ovary, spleen, liver, kidneys, lungs, heart, proventriculus and adrenals. Occular lymphomatosis causes lymphocyte infiltration of the iris (making the iris turn grey), unequal size of the pupils, and blindness. In addition to gross pathology and histology, other advanced procedures used for a definitive diagnosis of Marek’s disease include immunohistochemistry to identify cell type and virus-specific antigens, standard and quantitative PCR for identification of the virus, virus isolation to confirm infections and serology to confirm/exclude infections.

There are three serotypes of MD virus. Virulent (disease causing) chicken isolates fall into serotype 1. Avirulent (not disease causing) chicken isolates fall into serotype 2. Serotype 3 designates the related avirulent virus that is commonly found in turkeys.

However, because vaccination does not prevent infection with the virus, the Marek’s Disease virus has evolved increased virulence and resistance to this vaccine. As a result, current vaccines use a combination of vaccines consisting of HVT and gallidherpesvirus type 3 or an attenuated MDV strain, CVI988-Rispens (ATCvet code: Q011AD03). VECTORMUNE® HVT IBD & SB1 is live Marek Disease vaccines containing a genetically engineered Marek Disease virus of serotype 3 (turkey Herpesvirus or HVT) expressing key protective Infectious Bursal Disease antigens and a serotype 2 (SB-1) Marek Disease virus.

**PRINCIPLE OF THE TEST**

The kit is based on an indirect enzymatic immunoassay (Indirect ELISA). The MDV antigens are coated on plates. When a sample serum contains specific antibodies against virus, they will bind to the antigen on plates. Unbound antibodies are washed and the bound antibodies detected by anti-chicken IgG-HRP conjugate. After an incubation and washing, TMB substrate is added to detect bound conjugate. An initial color (blue) is produced that is converted to yellow by the addition of stop solution that is read at 450 nm. The amount of color is directly proportional to the amount of anti-MDV IgG present in the sample. Unknown samples are compared to the supplied negative and positive controls and results expressed as −ve or +ve.

**MATERIALS AND EQUIPMENT REQUIRED**

**Equipments:** Constant temperature box or incubator, microtiter plate spectrophotometer (630 nm) and absorbent paper

**Micropipettors:** 50 μL, 100 μL and 1000 μL

**PRECAUTIONS AND SAFETY INSTRUCTIONS**

The Anti-Marek’s Disease Virus (MDV) ELISA Test 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. Applicable SDS, 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).**

http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

**SAMPLE Dilutions**

Serum or plasma or other biological fluids can be used. Samples can be stored frozen at ~20oC or below. Dilute all samples 1:101 in sample diluent.

**Reagent Preparation**

**Washing buffer:** dilute 10X concentrated washing buffer with deionized water at 1:10 (dilute 50 ml conc in 450 ml water). Store at 4oC.

All reagents must be at room temperature prior to use. Diluent controls, samples, wash buffer, and prepare required amounts of working substrate (see page 3).

**Expiration Date:** 6-12 months (printed on the kit box)

**Storage:** Store at 2-8 °C in the dark

Alpha Diagnostic Intl. (www.4adi.com) 920-130-MDV/150603A