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

**GENERAL INFORMATION**

Pasteurella multocida is a pathogenic gram-negative morontic, pericellulose bacillus belonging to the Pasteurellaceae family. It has been classified into three subspecies, five capsular serogroup (A, B, C, D, and F) and 16 serotypes. P. multocida is the most common cause of respiratory tract infections in mammals and birds, including fowl (such as pigeons, chickens, and turkeys) and swine. It causes hemorrhagic septicaemia in cattle and buffalo. Infection with P. multocida is a significant cause of respiratory disease in many animals. It is a highly contagious pasteurellosis of rabbits primarily affecting the upper respiratory tract. Other Pasteurellaceae species, such as Pasteurella pneumotropica, chronic rhinitis, and otitis media as well as multiple abscesses. P. multocida can also cause a zoonotic infection in humans, which typically results in boils or abscesses from domestic pets. Many mammals (including domestic cats and dogs) and birds harbor it as part of their normal respiratory microflora. Pasteurella multocida is one of the most common bacteria isolated from calves suffering from shipping fever pneumonia. Pasteurella is found throughout the environment and within the upper respiratory tract of cattle, but it usually does not cause disease in otherwise healthy animals.

P. multocida genome shows 129 lip-proteins that are secreted and located in the outer membrane. Proteins of H.37,K5, serogroup B and D has been found to be the major lipoprotein in the outer membrane of the P. multocida. Lipopolysaccharides are important for survival of the bacteria in the host. These lipopolysaccharides, serogroups A and D) has surface adhesins and iron acquisition proteins for attachment and invasion of the host and to survive in a hostile environment. Type IV fimbrial subunit protein (PMA, ~31 kda, serogroups A, B, D, and F) is being explored as a vaccine candidate especially for H7 in bovines and septicaemic pasteurellosis in sheep and goat. A highly conserved membrane protein protein (PM) may serve as 'signature protein' in developing diagnostic assay or as a recombinant subunit vaccine. Experimental murine and cattle vaccines have been used to increase antibodies. A recombinant subunit protein PM experimental vaccine has also been developed.

**INTENDED USE**

Goat or Sheep Anti-Pasteurella multocida IgG test is an indirect ELISA suitable for detecting antibody against P. multocida antigens in serum or plasma following an inducing tissue culture medium, may be validated for use. This kit is also suited to determine the antibody status of multiparous and primiparous in a paper towel before sample addition.

**METHOD**

**KIT CONTENTS**

P. multocida is a pathogenic gram-negative morontic, pericellulose bacillus belonging to the Pasteurellaceae family. It has been classified into three subspecies, five capsular serogroup (A, B, C, D, and F) and 16 serotypes. P. multocida is the most common cause of respiratory tract infections in mammals and birds, including fowl (such as pigeons, chickens, and turkeys) and swine. It causes hemorrhagic septicaemia in cattle and buffalo. Infection with P. multocida is a significant cause of respiratory disease in many animals. It is a highly contagious pasteurellosis of rabbits primarily affecting the upper respiratory tract. Other Pasteurellaceae species, such as Pasteurella pneumotropica, chronic rhinitis, and otitis media as well as multiple abscesses. P. multocida can also cause a zoonotic infection in humans, which typically results in boils or abscesses from domestic pets. Many mammals (including domestic cats and dogs) and birds harbor it as part of their normal respiratory microflora. Pasteurella multocida is one of the most common bacteria isolated from calves suffering from shipping fever pneumonia. Pasteurella is found throughout the environment and within the upper respiratory tract of cattle, but it usually does not cause disease in otherwise healthy animals.

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**PRINCIPLE OF THE TEST**

The Anti-P. multocida IgG ELISA kit is based on the binding of antibody in samples to PM antigens coated on the plate, and virus antibody is detected by anti-PM IgG conjugate (spartic and isotype specific). After a washing step, substrate (TMB) is added color is directly proportional to the amount of antibody bound to antigens in the sample. Stop solution is added to terminate the reaction (converts blue to yellow color), and 455nm is then measured by the ELISA reader. The presence or concentration of antibody in samples is determined relative to supplied controls or calibrators.

**ASSAY DESIGN AND SET-UP**

Sample Collection and Handling: Serum, Plasma (EDTA, Citrated, Heparin) and other biological fluids are suitable with samples to detect antibody to avoid solution matrix interference. For serum, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. For other samples, clarify the sample by centrifugation 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.

**Antibody Stability and sample 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 test dilutions (1:100 or more) which would require prolonged low temperature should be done the same day as the assay. Do not store test dilution if necessary.

**Example:**

Initial dilution: 1:10 dilution serum + 90 ul WSD [or 0.1 ml + 90 ml WSD]
Further test dilution (1/20): 10 ul initial (1/10) + 190 ul WSD

**Review Calculation of Results (page 5) and Limits of the Assay before proceeding:**

- Select the proper sample dilutions accounting for expected potency of positives and minimizing nonspecific binding and other matrix effects; for example, net signal for non-immunized sera should be lower than the calibrator B (10 Ul) or user specified cut-off values. This is usually 1/10 or greater dilution for sera.
- Run a Sample Diluent Blank. This is an signal of producer-specific oligoantigen or antibody, especially of washing efficiency, and is used for for OD cutoff values. If required. Blank OD should be <0.3.

**Plate Setup**

Bring all reagents to room temperature (18°C-30°C) equilibration (at least 30 minutes).
- Determine the number of wells for the assay run. Duplicates are recommended, including 4 Control wells and 2 wells for each sample and internal control to be assayed.
- Remove the appropriate number of microwell strips from the pack and return unused strips to the pack. Reassemble the pack and store refrigerated.
- Remove the appropriate number of microwell strips from the pack and return unused strips to the pack. Reassemble the pack and store refrigerated.

**Materials Required But Not Provided:**

- Pipettes and pipettes that deliver 100ul and 1ul. A multi-channel pipette is recommended.
- Disposable propylene based no. 1 tubes for diluting samples and Anti-Mouse IgG HRP Concentrate.
- Graduated cylinder to dilute Wash Concentrate, 0.2 to 11.
- Stock bottle to store diluted Wash Solution, 0.2 to 1L
- Distilled or deionized water to dilute reagents.

**Assay Procedure**

- Add 200-300ul Working Wash Solution to each well and let stand for about 5 minutes. Aspirate or dump the liquid and place paper towel before sample addition.

**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 tissue culture medium. 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 tissue culture medium. 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 tissue culture medium.
**INTERPRETATION OF RESULTS**

<table>
<thead>
<tr>
<th>Wells</th>
<th>Std/samples</th>
<th>OD U/ml</th>
<th>Mean A450</th>
<th>Net A450</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1, A2</td>
<td>Blank (0 U/ml)</td>
<td>0.0</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>B1, B2</td>
<td>Calibrator A (3 U/ml)</td>
<td>3</td>
<td>0.200</td>
<td>0.19</td>
</tr>
<tr>
<td>C1, C2</td>
<td>Calibrator B (10 U/ml)</td>
<td>10</td>
<td>0.5</td>
<td>0.49</td>
</tr>
<tr>
<td>D1, D2</td>
<td>Calibrator C (30 U/ml)</td>
<td>30</td>
<td>1.09</td>
<td>1.08</td>
</tr>
<tr>
<td>D1, D2</td>
<td>Calibrator D (100 U/ml)</td>
<td>100</td>
<td>2.17</td>
<td>2.16</td>
</tr>
</tbody>
</table>

The initial dilution of the samples (1:100) has been taken into consideration when reading the results from the graph. Therefore, antibody concentration of the samples can be directly read using the standard curve. Samples showing concentration above the highest standard have to be re-tested at a dilution of 1:400 or higher. The result in U/ml read from the calibration curve for this sample must then be multiplied by a factor of 4.

**Cut-Off Values**

Samples tested at 1:100 dilution and yielding values >calibrator B (10 U/ml) may be considered positive. These cut-off values are not universal and users are encouraged to establish their own cut-off values that is representative of the given animal population (Age, sex, and exposure to the pathogens).

**Assay Sensitivity**

The antigen coating level, HRP conjugate concentration, and sample Diluent are optimized to differentiate anti-PM IgG from background (non-antibody) signal with serum samples at an appropriate dilution. The positive controls at 100 U/ml represent about 100 ng/ml anti-PM Multocida IgG. The lowest limit of detection is about 0.3 ng of IgG.

**Limitation of the Assay**

- The assay detects and quantifies IgG antibodies directed to the P. multocida antigens. It may be possible for an animal to have antibodies without clinical systems.
- Anti-P. multocida antibody levels of an infected animal may be below detection threshold related to the time course of the infection, e.g., too early for positive titer development.
- This kit does not identify the antibodies from various P. Multocida strains.

**Quality Control**

Standards must be found within the acceptable ranges. Blanks must not exceed >3.00 and the high std must be >1.00. Repeat the test for significant deviations and report to ADI. We strongly recommend running internal reference controls in each test. No single negative or cut-off may represent the entire population of porcine samples as the animal habitat and exposure to the virus varies, therefore, basal level of anti-P. multocida antibodies will change in any given population.

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**Antibody Titers from standard Curves**

**Goat/Sheep Anti-Pasteurella multocida IgG (Anti-PM IgG) ELISA kit**

For the detection of P. Multocida IgG in Serum, plasma or other biological fluids.

For in vitro research use only (RUO), not for therapeutic or diagnostic use.

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**Goat/Sheep Anti-Pasteurella multocida IgG (Anti-PM IgG) ELISA kit**

Cat. #: AE-310840-1, 96 tests

Alpha Diagnostic Intl [www.4adi.com]

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**PRODUCT SPECIFICATIONS**

**Specifity**

Highly purified P. multocida antigens are used to coat the microwells; thus the assay is specific for antibodies directed to P. multocida. Antibodies from various strains may crossreact with the antigens used in the kit. The Anti-pasteurella IgG HRP conjugate reacts specifically with goat/sheep IgG class antibodies. IgM and IgA antibody would not be measured above background signals. Antibodies to P. multocida recombinant proteins (P/PA, V/AC) may provide more specific detection than the use of whole P. multocida antigens in this assay.

**P. multocida in ruminants**

Respiratory tract infections are of common occurrence in various species of domestic animals. However, pneumonic pasteurellas, also known as respiratory manehemovirus, is most common example with a wide prevalence in ruminants and accounts for approximately 35% of the total cattle deaths worldwide (Baundreaux, 2004). Pasturella multocida is associated with haemorrhagic septicaemia and enzootic pneumonia complex in sheep, goats, and cattle and buffaloes (Jones 1997). Normally, inhaled bacteria like Pasturella is killed and removed by the body’s antibodies and macrophages. Pasteurella can cause disease when it is inhaled into the deeper portions of the respiratory tract and the animal’s normal defense system is impaired. P. multocida A3 is the most common serotype isolated from SBD with antibody prevalence of up to 90% depending upon the region.

**References:**