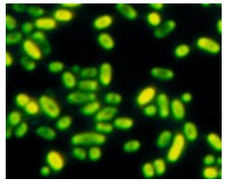
INTENDED USE

Mouse Anti-Pasteurella multocida IgG test is an indirect ELISA suitable for detecting antibody against P. multocida antigens in serum or plasma. Other biological fluids, including tissue culture medium, may be validated for use. This kit is also suited to determine the efficacy of P. multocida vaccines. *For in vitro research use only (RUO), not for therapeutic or diagnostic use*.

GENERAL INFORMATION



Pasteurella multocida is a pathogenic gram-negative nonmotile, penicillin-sensitive coccobacillus belonging to the Pasteurellaceae family that has been classified into three subspecies, five capsular serogroup (A, B, D, E, and F) and 16 serotypes. P. multocida is the cause of a range of **diseases in mammals and birds, including fowl cholera in poultry, atrophic rhinitis in pigs, and bovine hemorrhagic septicemia in cattle and buffalo**. Infection with P. multocida is a significant cause of clinical disease in rabbits. Snuffles, a highly contagious **pasteurellosis** of rabbits primarily affects the upper respiratory tract with potential fatal consequences, such as septicemia, pneumonia, chronic rhinitis, and otitis media as well as multiple abscesses. P. multocida can also cause a zoonotic infection in humans, which typically is a result of bites or scratches from domestic pets. Many mammals (including domestic cats and dogs) and birds harbor it as part of their normal respiratory microbiota. 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 lipo-proteins that are secreted and located in the outer membrane. **Protein H (37.5 kda,** serogroup **D)** has been found to be the major polypeptide in the outer membrane of the *P. multocida*. **Lipopolysaccharides** are important for survival of the bacteria in the host. The ***P. multocida* toxin (PMT, 146 kda,** serogroups **A and D)** has surface adhesins and iron acquisition proteins for attachment and invasion of host cells and to survive in a hostile environment. Type IV fimbrial subunit protein (**Ptfa**, ~31 kda, serogroups A, B, D, and F) is being explored as a vaccine candidate expecially for HS in bovines and septicaemic pasteurellosis in sheep and goat. A highly conserved outer membrane protein, **Vacj ~26 kda**, may serve as 'signature protein' in developing diagnostic assay or as a recombinant subunit vaccine.

PMT Vaccines: **Live bacterial cultures** vaccination have been used to increase antibodies. **A recombinant subunit protein H** experimental **vaccine** has also been developed.

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 antibody-HRP conjugate (species and isotype specific). After a washing step, substrate (TMB) is added and color (blue) is developed, which is directly proportional to the amount of antibody present in the sample. Stop Solution is added to terminate the reaction (converts blue to yellow color), and A450nm is then measured using an ELISA reader. The presence or concentration of antibody in samples is determined relative to supplied controls or calibrators.

KIT CONTENTS

The microtiter well plate and all other reagents, if unopened, are stable at 2-8^{O}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 (50x) Cat. No. WB-50, 10ml	Dilute the entire volume 20ml + 980ml with distilled or deionized water into a clean stock bottle. Label as **Working Wash Solution** and store at ambient temperature until kit is used entirely.
Sample Diluent Concentrate (20x) Cat. No. SD20B-SP, 10ml, (pink solution)	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as **Working Sample Diluent** (WSD) and store at 2-8ºC until the kit lot expires or is used up.
Anti-Mouse IgG-HRP Conjugate Concentrate (100x) Part: 310823, 0.11ml	in buffer with detergents and antimicrobial as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of **Working Sample Diluent** is sufficient for 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
PMT antigen Microwell Strip Plate	310811	8-well strips (12)	Coated with PMT antigen, and post-coated with stabilizers.
3 U/ml 10 U/ml 30 U/ml 90 U/ml	310812A 310812B 310812C 310812D	0.65 ml 0.65 ml 0.65 ml 0.65 ml	Four (4) vials, each containing anti-PMT IgG levels in arbitrary activity Units; diluted in buffer with protein, detergents and antimicrobial as stabilizers.
Mouse anti-P. multocida IgG +ve control	310820-PC	0.65 ml	containing anti-PMT IgG levels in arbitrary activity Units (lot specific concn on the vial)
TMB Substrate	80091	12 ml	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-Mouse IgG HRP Concentrate.
- Graduated cylinder to dilute Wash Concentrate; 0.2 to 1L.
- Stock bottle to store diluted Wash Solution; 0.2 to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- Microwell plate reader at 450 nm wavelength.

ASSAY DESIGN AND SET-UP

Sample Collection and Handling

Serum, Plasma (EDTA, Citrated, Heparin) 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. For other samples, clarify the sample by centrifugation and/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 provides the lowest assay background should be done the same day as the assay. Do not store test dilution. If necessary, use the

Example:
Initial (1/10): **10**ul serum + **90**ul WSD [or 0.1ml + 0.9ml]
Further test dilution (1/20): **20** ul initial (1/10) + **180** ul WSD

Assay Design

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 non-specific binding and other matrix effects; for example, net signal for non-immune samples should be lower than the **calibrator B (10 U/ml) or user specified cut-off values.** This is usually 1/200 or greater dilution for sera.
- 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.

Plate Set-up

Bring all reagents to room temperature (18-30º 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 pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
- Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.

Assay Procedure

- 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.

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 [50 or 100ul – 60 min; 4 washes]

- Add 100ul of WSD (blank), 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 Antibody-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 within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.

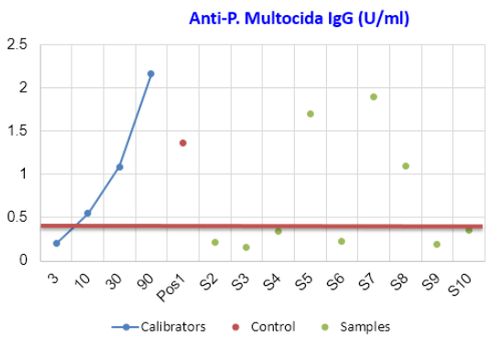
PRECAUTIONS AND SAFETY INSTRUCTIONS

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: Sample Diluent and anti-Protein G-HRP contain Proclin 300 (0.05%, v/v). http://4adi.com/objects/catalog/product/extras/ELISA-Kit-SDS-MSDS-Set-1.pdf

INTERPRETATION OF RESULTS

Wells	Stds/samples	U/mL	Mean A450	Net A450
A1, A2	**Blank (0 U/ml)**	0.0	0.009	-
B1, B2	Calibrator A (3 U/ml)	3	0.200	0.19
C1, C2	Calibrator B (10 U/ml)	10	0.5	0.49
D1, D2	Calibrator C (30 U/ml)	30	1.09	1.08
D1, D2	Calibrator D (100 U/ml)	100	2.17	2.16

Antibody Titers from standard Curves



A typical standard curve. Do not use for calculation of sample values. (Pos1=positive control; S2-S10; samples tested at 1:100 dilutions; Red line is the suggested cut-off).

2/soum/AE-310820/ELISA-graph

Calculations

1. Subtract blank values from all values (standards, controls and samples) to calculate the net A450.
2. 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).
3. 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
   = **Total IgG Antibody Activity Units**

The initial dilution of the samples (1:200) 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 concentrations 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 Mouse IgG. The lowest limit of detection is about 0.3 ng of Mouse IgG.

Limits 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.

Quality Control

Standards must be found within the acceptable ranges. Blanks must not exceed >0.300 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 world 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.

PRODUCT SPECIFICATIONS

Specificity

Highly purified P. multocida antigens are used to coat the microwells; thus the assay is specific for antibodies directed to P. multocida. The Anti-Mouse IgG HRP conjugate reacts specifically with Mouse IgG class antibodies; IgA, IgM and IgE antibody would not be measured above background signals. Antibodies to P. multocida recombinant proteins (PTFA, VacJ) may provide more specific detection than the use of whole P. multocida antigens in this assay

P. multocida in animals

Most mammals can be infected with P. multocida. It is most common in rabbits. It is also common in dogs, cats, livestock but is rare in rodents (mice and rats). Normally, inhaled bacteria like Pasteurella 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.

References: Baker DG (2003) in natural pathogens of lab animals; their effect on research, ASM Press, 385pp; Pati US (1996) Vet. Microbiol. 52, 301-311; Ruffalo GC (1997) Inf. Immunity 65, 339-343; Qureshi S (2014) Vet. World 7, 224-228; Shivachandra, SB (2014) Vaccine 32, 290-296; Tabatabai, LB (2004) Infections and Immunity. 72: 1195–1198; Syuto B (1982) Infections and Immunity, 37: 1218-1226; Ahmad AM (2014) Science World J. 9, 1-7.

Related Items

Catalog#	Product Description
AE-310800-1	Rabbit Anti-P. multocida IgG ELISA Kit,
AE-310805-1	Chicken Anti- P. multocida IgG ELISA Kit,
AE-310810-1	Bovine Anti-P. multocida IgG ELISA Kit,
AE-310815-1	Porcine P. multocida IgG ELISA Kit
AE-310820-1	Mouse/Rat Anti- P. multocida IgG ELISA
AE-310825-1	Human Anti- P. multocida IgG ELISA Kit,
AE-310830-1	Monkey Anti- P. multocida IgG ELISA Kit,
PMT11-S	Anti-P. multocida toxin (PMT) antiserum
PMT15-N-10	Purified Anti- P. multocida Toxin (PMT, 146 kda, >95%)
PMUL11-S	Anti- P. multocida antigens antiserum
PTFA11-A	Anti-P multocida Type IV fimbrial subunit protein (PTFA) P. IgG, aff pure
PTFA16-P	Anti- P. multocida Type IV fimbrial subunit protein (PTFA) control peptide
VacJ11-S	Anti- P. multocida virulence associated chromosome locus J (Vacj) protein antiserum

Instruction Manual No. AE-310820-1

Mouse Anti-Pasteurella multocida IgG (Anti-PM IgG) ELISA kit

Cat. #. AE-310820-1, 96 tests

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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