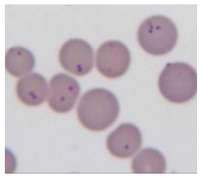
INTENDED USE

Mouse Anti-Mycoplasma pulmonis (Mp)IgG tests an indirect ELISA suitable for detecting antibody against Mp in serum or plasma. Other biological fluids, including tissue culture medium, may be validated for use *in vitro research use only (RUO), not for therapeutic or diagnostic use*.

GENERAL INFORMATION



Mycoplasma is a Gram negative bacteria that lack a cell wall around their cell membrane. Mycoplasma species are the smallest bacterial cells yet discovered. Without a cell wall, they are unaffected by many common antibiotics such as penicillin or other beta-lactam antibiotics that target cell wall synthesis. They can be parasitic or saprotrophic. Several species are pathogenic in humans, including ***M. pneumoniae***, and *M. genitalium*, which is believed to be involved in pelvic inflammatory diseases. **Mycoplasma Pulmonis(Mp)**is a pleomorphic bacteria that causes illness (pneumonia) primarily in rats and mice, though guinea pigs are susceptible to experimental infection. It primarily colonizes the middle ear and nasopharynx, causing a pneumonia-like illness in its host. Symptoms may include ruffled fur, reluctance to move, weight loss, and reproductive changes. *M. Pulmonis* disseminates widely throughout the host affecting a number of organ systems, thereby rendering infected animals unfit for certain research. Over **100 species** have been included in the genus Mycoplasma. The genus Mycoplasma uses vertebrate hosts. The severity of lesions in respiratory tissues and mortality due to MRM appears to be species and strain dependent. C57BL/6 mice are less prone to *M. pulmonis* infection than C3H/He and DBA/2 mice. Lewis rats are more susceptible to *M. pulmonis* than F344 rats. Mycoplasma species are often found in research laboratories as contaminants in cell culture. An estimated 11 to 15% of U.S. laboratory cell cultures are contaminated with mycoplasma. European labs and other countries contamination rates are higher (up to 80%). Mycoplasma pulmonis genome (963.8Kb) contains 782 putative coding sequences (CDSs).

To diagnose infection, clinical symptoms may be identified for advanced stage mycoplasmosis. Colony surveillance can be accomplished by immunofluorescent antibody tests (IFA), polymerization chain reaction (PCR), and enzyme-linked immunosorbent assay (ELISA). Culture of *M. Pulmonis* may be performed, although the organism has slow growth capabilities.

PRINCIPLE OF THE TEST

The Mouse Anti-Mp IgG ELISA kit is based on the binding of mouse antibody in samples to M.pulmonis antigen coated on the plate, and virus antibody is detected by anti-mouse IgG-specific antibody conjugated to HRP. 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. #WB-50, 10 ml	Dilute the entire volume 10ml + 490 ml with distilled or deionized water into a clean stock bottle. Label as ***1X*WashSolution** and store at 4^{o}C for long term and ambient temp.for short term.
Sample Diluent Concentrate (20x) Cat. No. SD-20T, 10ml	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as **Working Sample Diluent** and store at 2-8^{o}C until the kit lot expires or is used up.
Anti-MouseIgG-HRP Conjugate Concentrate (100x) Part: 300704, 0.15ml	Peroxidase conjugated anti-MouseIgGin buffer with detergents and antimicrobial as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of **Working Sample Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return 100X to 2-8^{o}C storage.

Ready For Use: Store as indicated on labels.

Component	Part	Amt	Contents
Mp antigen Microwell Strip Plate	310501	8-well strips (12)	Coated withMpantigen, and post-coated with stabilizers.
Mouse Anti-Mp IgGSensitivity Control	310502	0.65 ml	**Low** level mouse anti-Mp in buffer with detergents and antimicrobial.
Mouse Anti-Mp Positive Control (100 U/ml)	310503	0.65 ml	**High** levelmouse anti-M. pulmonis, in buffer with detergents and antimicrobial.
TMB Substrate	80091	12 ml	Chromogenic 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-MouseIgGHRP 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 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(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 (1/10): **20** ul initial (1/10) + **180**ul WSD

Assay Design
Review Calculation of Results (p5-7) and Limits of the Assay (above) before proceeding:

- Select the proper sample dilutions accounting for expected potency of positives and minimizing non-specific binding (NSB) and other matrix effects; for example, net signal for non-immune samples should be lower than the **SensitivityControl**. This is usually 1/100 or greater dilution for mouse sera with normal levels of IgG and IgM.
- 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.
- Run the **Positive** and **SensitivityControls**, which validate that the assay was performed to specifications: the **Positive** Control should give a high signal (>0.8 OD); the **Sensitivity** Control should give a low signal which can be used to discriminate at the Positive/Negative threshold (see Interpretation of Results, p. 5).

Plate Set-up
Bring all reagents to room temperature (18-30^{o} C) equilibration (at least 30 minutes).

- Determine the number of wells for the assay run. Duplicates are recommended, including 4Control 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 Anti-MouseIgGHRP 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

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

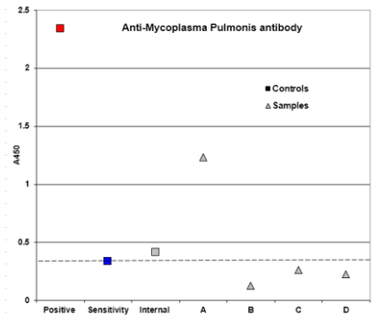
INTERPRETATION OF RESULTS

A. Antibody Activity Threshold Index

Compare Samples to **Sensitivity Control** or **Internal Control**

=Positive/Negative Cut-off.

Example:



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Results

The **sensitivity** of the assay to detect anti-M. pulmonisIgG, from either natural infection or MAP, is controlled so that the **Sensitivity Control** represents a threshold OD for most true positives in mouse serum at 1:100 or greater. Visual inspection of the data in the above graph shows the following:

Positive Control – clearly positive, shows the OD range of the assay; high value indicates optimal sensitivity of the assay.

Sensitivity Control – a 'Cut-off' line has been drawn to indicate a threshold distinguishing between**Positive/Negative**. This not a clear-cut threshold, rather a low OD area that could represent either low positives or high background negatives.

Internal Control – a true positive from an infected animal that represents the lab's experience in distinguishing low positive from negative samples. This should be run in each assay to supplement the Sensitivity Control for Positive/Negative discrimination purposes.

Samples A,B,C,D – 3 samples (B, C, D) are negative: below the threshold; 1 sample (A) is positive: clearly above the threshold.

The Sensitivity Control can be used to calculate a **ThresholdIndex**that numerically discriminates Positive/Negative, as follows:

- Divide each Sample net OD by the Sensitivity Control net OD. Values above 1.0 are a measure of **Positive** Antibody Activity; below 1.0 are **Negative** for antibody.

This calculation was used to represent Assay Precision, page 7.

INTERPRETATION OF RESULTS (cont)

Antibody Titers from positive control dilution Curves

Positive control has been assigned an arbitraryM. pulmonis IgG activity of 100 U/ml. Prepare 2-fold serial dilutions of the supplied control with the 1XWSD and prepare additional calibrators at 50, 25, 12, 6 U/ml. Run additional control and plot a standard curve to calculate unknown values.

The titer of antibody activity calculated from a dilution curve of each sample is recommended as the most accurate quantitative method for animals immunized with M. pulmonis antigens. Best precision can be obtained using the following guidelines:

1. Use an OD value Index in the mid-range of the assay (2.0 – 0.5 OD); this provides the best sensitivity and reproducibility for comparing experimental groups and replicates. An arbitrary 1.0 OD is commonly used.

2. Prepare serial dilutions of each sample to provide a series that will produce signals higher and lower than the selected index. With accurate diluting, duplicates may not be required if at least 4 dilutions are run per sample.

3. A 5-fold dilution scheme is useful to efficiently cover a wide range which produces ODs both above and below 1.0 OD. The dilution scheme can be tightened to 3-fold or 2-fold for more precise comparative data.

4. The Positive, Negative and/or Internal Controls can be used to normalize inter-assay values.

Calculations

1. 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).

2. 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**

Note:

- The sensitivity of the assay may be increased to perhaps convert a borderline sample to a positive by using a lower dilution of the sample, e.g., 1/50. The values of negatives may increase, so an alternative threshold should be established using known negatives to develop a **Positive Index** (page 6), or by using known **Internal Controls** as discriminator for a **Threshold Control** (instead of the kit Sensitivity Control)

Limits of the Assay

- The assay detects and quantifies IgG antibodies directed to the spike (S) glycoprotein. It may be possible for an animal to have Mouse Hepatitis Viral infection without producing antibodies specific to the S glycoprotein.
- Anti-MHV 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.

PRODUCT SPECIFICATIONS

Specificity

Highly purified M. pulmonis antigens are used to coat the microwells; thus the assay is specific for antibodies directed to M. pulmonis. The Anti-Mouse IgGHRP conjugate reacts specifically with mouse IgG class antibodies; IgA, IgM and IgE antibody would not be measured above background signals.

Over **100 species** have been included in the genus Mycoplasma. Many antigens share substantial protein sequence homology with within the mycoplasma family. So most ELISA kits using Mp antigens may detect many cross-reactive mycoplasma strains.

Assay Sensitivity

The Mp antigen coating level, HRP conjugate concentration, and sample Diluentare optimized to differentiate anti-M. pulmonisIgG from background (non-antibody) signal with mouse 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.

References: McAuliffe L (2006) Microbiol. 152, 913-922; Reyes L (2000) Comp. Med. 50, 622-627; Sandstedt K (1997) Clin. Exo. Immunol. 108, 490-496; Shoeb TR (1987) Vet. Pathol. 24, 392-399; Yancey AL (2001) Inf. Imm. 69, 2865-2871

Related Items

Catalog#	Product Description
AE-310500-1	Mouse Anti-Mycoplasma Pulmonis (Mp) IgG ELISA Kit, 96 tests
AE-310500-NC	Mouse Anti-Mp IgG -ve serum
AE-310500-PC	Mouse Anti-M) IgG +ve serum
AE-310510-1	Rat Anti-Mp IgG ELISA Kit, 96 tests
AE-310510-NC	Rat Anti-Mp IgG -ve serum
AE-310510-PC	Rat Anti-Mp IgG +ve serum
AE-310520-1	G. pig Anti-Mp IgG ELISA Kit, 96 tests
AE-310520-NC	G. pig Anti-Mp IgG -ve serum
AE-310520-PC	G. pig Anti-Mp IgG +ve serum
MPUL11-S	Anti-Mp IgG (all antigens)
MPUL15-N-100	Mp protein antigens (mammalian cell)

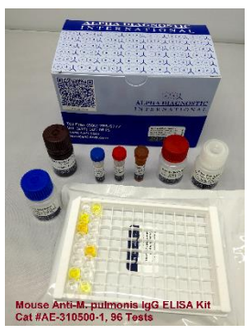
Instruction Manual No. AE-310500-1

Mouse Anti-Mycoplasma pulmonis (Mp) IgG ELISA kit

Cat. #. AE-310500-1

For the detection of Mycoplasma pulmonis (Mp) IgG in Serum or plasma

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



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ELISA Kit Components	Amount	Part
Mp antigens Coated Strip Plate	8-well strips	310501 (12
Mouse Anti-Mp IgG Sensitivity Control	0.65ml	310502
Mouse Anti-Mp Positive Control	0.65 ml	310503
Anti-Mouse IgG HRP Conjugate (100X)	0.15 ml	300704
Sample Diluent (20x)	10 ml	SD20T
Wash Solution Concentrate (50X)	10 ml	WB-50
TMB Substrate	12 ml	80091
Stop Solution	12 ml	80101
Product Manual	1 ea	M-AE-310500-1