GENLISA™ Rat Mycoplasma Pulmonis Antibody IgG ELISA

REF : KLR300

Ver 1.7

RUO

Immunoassay for Qualitative Determination of Mycoplasma Pulmonis Antibody IgG ELISA in Rat serum, plasma, other biological samples.

RUO	**For Research Use Only**	**REF**	**Catalog Number**
	Store At	**LOT**	**Batch Code**
	Manufactured By		**Biological Risk**
	Expiry Date		**Consult Operating Instructions**

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REF KLR300 Σ 96 tests

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Introduction:

The GENLISA™ ELISA kits are used for assessing the specific biomarker in samples analytes which may be serum, plasma and cell culture supernatant as validated with the kit. The kit employs a sandwich ELISA technique which leads to a higher specificity and increased sensitivity compared to conventional competitive ELISA kits which employ only one antibody. Double antigens are used in this kit.

Intended Use:

The GENLISA™ Rat Mycoplasma Pulmonis Antibody IgG ELISA is used as an analytical tool for qualitative determination of Rat Mycoplasma Pulmonis Antibody IgG in serum, plasma and other biological samples.

Principle:

The method employs sandwich ELISA technique. Rat Mycoplasma Pulmonis antigen is pre-coated onto microwells. Samples and controls are pipetted into microwells and Rat Mycoplasma Pulmonis Antibody IgG present in the sample are bound by the antigens. Then a Horseradish Peroxidase (HRP)-antigen conjugated is pipetted and incubated to form a complex. After washing microwells in order to remove any non-specific binding, the substrate solution (TMB) is added to microwells and color develops proportionally to the amount of Rat Mycoplasma Pulmonis Antibody IgG ELISA in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Rat Mycoplasma Pulmonis Antigen Coated Microtiter plate – 96 wells
2. Negative Control – 0.5 ml
3. Positive Control – 0.5 ml
4. Mycoplasma Pulmonis: HRP Conjugate – 10 ml
5. Sample Diluent – 6 ml
6. (20X) Wash Buffer– 25 ml
7. TMB Substrate – 12 ml
8. Stop Solution – 12 ml
9. Instruction Manual

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul
3. Deionized (DI) water
4. Wash bottle or automated microplate washer
5. Graph paper or software for data analysis
6. Timer
7. Absorbent Paper

Handling/Storage:

1. All reagents should be stored as indicated on the component label.
2. All the reagents and wash solutions should be used within 12 months from manufacturing date.
3. Before using, bring all components to room temperature (18-25ºC). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.
4. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
2. For Research Use Only.

Specimen Collection and Handling:

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

1. The kit cannot test samples which contain NaN3, because NaN3 inhibits HRP activity.
2. Extract as soon as possible after specimen collection as per relevant procedure. The samples should be tested as soon as possible after the extraction. Alternately the extracted samples can be kept in -20°C. Avoid repeated freeze-thaw cycles.
3. **Serum**- Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, re-centrifuge.
4. **Plasma**- Use EDTA or citrate plasma as an anticoagulant, mix for 10-20 minutes; centrifuge for 20-min at the 2000-3000 rpm. Remove the supernatant. If precipitation appears, re-centrifuge.
5. **Cell samples** - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20^0C or -80^0C. Avoid repeated freeze-thaw cycles.

Sample Preparation: Add 10 ul of sample and to this add 40 ul of sample diluent, mix well with gently shaking. Samples should be loaded onto the bottom without touching the well wall.

Reagent Preparation (all reagents should be diluted immediately prior to use):

1. Allow all components to reach RT (Room Temperature) prior to use in the assay.
2. To make Wash Buffer (1X); dilute 25 ml of (20X) Wash Buffer in 475 ml of DI water.

Procedural Notes:

1. Read all the instructions thoroughly before performing the test.
2. Allow all reagents to reach Room Temperature before beginning and reconstitute or dilute the required reagents.
3. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess unreacted reagents is essential.
4. All Controls and Samples should be assayed at least in duplicates.
5. The assay has been optimized to be used with the protocol mentioned. Any deviation from the same may invalidate the results.

Assay Procedure:

1. It is strongly recommended that all Controls and Samples be run in duplicates or triplicates. All steps must be performed at 37°C.

2. Pipette **50 ul of Positive Control, Negative Controls** and **Diluted Samples** into the respective wells.

3. Add **100 ul of Mycoplasma Pulmonis: HRP Conjugate** into each well.

4. Cover the plate and incubate for 60 minutes at 37ºC.

5. Aspirate and wash plate 5 times with Wash Buffer (1X) and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step.

6. Add **100 ul of TMB Substrate** in each well.

7. Incubate the plate at 37ºC for 15 minutes in dark. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.

8. Pipette out **100 ul of Stop Solution**. Wells should turn from blue to yellow in color.

9. Read the absorbance at 450 nm with a microplate within 10-15 minutes after addition of Stop solution.

Calculation of Results:

Determine the Mean Absorbance for each set of duplicate Controls and Samples. Results are interpreted qualitatively by calculating a cut-off value for each sample on the basis of the cut-off determined. Read Absorbance at 450nm with an ELISA reader.

Cut-Off value (CO) = OD_{mean} of Negative Control + 0.15

Validity of the test:

The test is valid if the following conditions are met,
Mean Absorbance of Negative Control ≤ 0.15
Mean Absorbance of Positive Control ≥ 1.00

Interpretation of Results:

Negative Results: if the OD value < CUT OFF, the sample is Negative for Mycoplasma Pulmonis Antibody IgG.

Positive Results: if the OD value ≥CUT OFF, the sample Positive for Mycoplasma Pulmonis Antibody IgG.

Safety Precautions:

- **This kit is For Research Use only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from Rat body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed

- Do not smoke, eat or drink while handling kit material
- Always use protective gloves
- Never pipette material by mouth
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.

In any case GLP should be applied with all general and individual regulations to the use of this kit.

LIMITED WARRANTY

Krishgen Biosystems does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the Products; against defects in products or components not manufactured by Krishgen Biosystems, or against damages resulting from such non-Krishgen Biosystems made products or components. Krishgen Biosystems passes on to customer the warranty it received (if any) from the maker thereof of such non Krishgen made products or components. This warranty also does not apply to

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THANK YOU FOR USING A KRISHGEN PRODUCT!

SYMBOLS KEY

MTP	Coated Microtiter Plate (96 wells)
PC	Positive Control
NC	Negative Control
HRP CONJ	Mycoplasma Pulmonis :HRP Conjugate
SAMP DIL	Sample Diluent
20X WASH BUF	(20X) Wash Buffer
SUB TMB	TMB Substrate
SOLN STOP	Stop Solution
	Consult Instructions for Use
REF	Catalog Number
	Expiration Date
	Storage Temperature