GENLISA Canine Brucella Antibody IgG ELISA

REF : KAD5013

Ver 1.0

PackSize: 96 wells

For Research Use Only

Enzyme Immunoassay for the estimation of IgG Antibodies to Brucella in Canine serum, plasma and other biological fluids

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

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 competitive ELISA kits which employ only one antibody.

Intended Use:

Enzyme Immunoassay for the estimation of Canine Brucella IgG Antibodies in serum, plasma, tissue homogenates and other biological fluids as validated.

Principle:

The method employs a sandwich enzyme-linked immunosorbent assay (ELISA) technique to assay the level of the analyte in samples. Standards or Samples are captured by the immobilized antibody in the microtitre plate wells. Wells are washed to remove any unbound standards and analyte present in the sample. A biotinylated protein is added to form a complex with the analyte and the antibody. Wells are washed to remove the excess conjugate and Streptavidin:HRP Conjugate is added to the microplate and incubated. After incubation and a washing step TMB Substrate, are added. Blue color develops on incubation and the reaction is stopped with a Stop Solution to form a yellow color. The concentration of the analyte in the samples is directly proportional to the yellow color developed (absorbance) in the wells

Materials Provided:

1. Coated Microtiter Plate (12 x 8 wells) - 1 no
2. Controls (Positive, Negative) - 2 vials
3. HRP Conjugate (concentrated) - 120 ul
4. Sample Diluent - 20 ml
5. Antibody Dilution Buffer - 10 ml
6. HRP Conjugate Dilution Buffer - 12 ml
7. (25X) Wash Buffer - 20 ml
8. TMB Substrate - 12 ml
9. Stop Solution - 12 ml
10. 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. Clean tubes and Eppendorf tubes.
6. Precision single and multi-channel pipette and disposable tips.
7. 37°C incubator.
8. Timer.

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.

Sample Preparation and Storage:

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

1. 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.
2. **Serum-** Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.
3. **Plasma-** Use EDTA or citrate plasma as an anticoagulant, mix for 10-20 minutes; centrifuge for 15-min at 2000-3000 rpm. Remove the supernatant carefully. If precipitation appears, recentrifuge.
4. **Tissue Samples-** Rinse tissues in PBS (pH 7.4) to remove excess blood thoroughly and weigh before homogenization. Mince tissues and homogenize them in PBS (pH7.4) with a glass homogenizer on ice. Thaw at 2-8°C or freeze at -20°C. Centrifuge at 2000-3000 RPM for approximately 20 minutes and collect the supernatant carefully.
5. **Cell Culture supernatants and other biological fluids-** Centrifuge samples at 1000 x g for 20 minutes. Collect the supernatant and assay immediately or store samples in aliquot at -20˚C or -80˚C for later use. Avoid repeated freeze-thaw cycles.

Note: *Grossly hemolyzed samples are not suitable for use in this assay.*

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

1. Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.
2. Bring all reagents to Room temperature before use.
3. To make **Wash Buffer (1X) 500 ml;** dilute **20 ml of (25X) Wash Buffer in 480 ml of DI water**.
4. **HRP Conjugate Working Solution**- Briefly spin or centrifuge the HRP Conjugate (concentrated) before use. Dilute them to the working concentration 100-fold with HRP Conjugate Dilution Buffer.

Procedural Notes:

1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
2. High Dose Hook Effect may be observed in samples with very high concentrations of the analyte.
3. Avoid assay of Samples containing sodium azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of analyte.
4. It is recommended that all Standards and Samples be assayed in duplicates or triplicates.
5. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
6. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to compromisation of the sensitivity of the assay.
7. The plates should be read within 30 minutes after adding the Stop Solution.
8. Make a work list in order to identify the location of Standards and Samples.

Assay Procedure:

1. It is strongly recommended that all Standards and Samples be run in duplicates or triplicates. A standard curve is required for each assay.

2. Add **50 ul prepared Standards.**

3. Add **40 ul Samples with 10 ul of Sample Diluent** to respective wells.

4. Cover the plate with a sealer and incubate for 30 minutes at 37°C.

5. Aspirate and wash plate 4 times with diluted 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. Pipette **50 ul HRP Conjugate Working Solution** to all wells except the blank wells. Mix well.
7. Cover the plate with a sealer and incubate for 30 minutes at 37°C.
8. Aspirate and wash as per Step (6) above.
9. Pipette **50 ul TMB Substrate** in all the wells.
10. Incubate the plate at **37°C** for **20 minutes**. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.
11. Pipette **50 ul** of **Stop Solution** to all wells. The wells should turn from blue to yellow in color.
12. 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 or triplicate Standards and Samples.

Test results are accounted as valid if the
1.average value of positive control ≥1.00.
2.average value of negative control ≤0.10.

The critical value (Cut Off) calculation:
Cut-Off Value = Mean Value of Negative Control + 0.15

Negative Result:
OD Value < Cut Off, the sample is Canine Brucella IgG negative.

Positive Result:
OD Value ≥ Cut Off, the sample is Canine Brucella IgG positive.

Quality Control:
It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

Performance Characteristics of the Kit:
This kit has been validated. Please view the details herein below.

Specificity:
This assay has high sensitivity and excellent specificity for detection of the target analyte. No significant cross-reactivity or interference between the analyte and analogues was observed.

Recovery
Matrices listed below were spiked with certain level of the analyte and the recovery rates were calculated by comparing the measured value to the expected amount of analyte in samples.

Matrix	Recovery Range (%)	Average (%)
serum(n=5)	87-99	93
EDTA plasma(n=5)	90-104	97
heparin plasma(n=5)	80-97	88

Precision:
Intra-Assay: CV<8%
Inter-Assay: CV<10%

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.
- Source materials maybe derived from Human 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 Private Limited 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 Private Limited, or against damages resulting from such non-Krishgen Biosystems Private Limited made products or components. Krishgen Biosystems Private Limited 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 Products to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Biosystems Private Limited.

THIS WARRANTY IS EXCLUSIVE. The sole and exclusive obligation of Krishgen Biosystems Private Limited shall be to repair or replace the defective Products in the manner and for the period provided above. Krishgen Biosystems Private Limited shall not have any other obligation with respect to the Products or any part thereof, whether based on contract, tort, and strict liability or otherwise. Under no circumstances, whether based on this Limited Warranty or otherwise, shall Krishgen Biosystems Private Limited be liable for incidental, special, or consequential damages.

This Limited Warranty states the entire obligation of Krishgen Biosystems Private Limited with respect to the Products. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

Krishgen Biosystems Private Limited. 2026

THANK YOU FOR USING A KRISHGEN PRODUCT!

KRISHGEN BIOSYSTEMS PRIVATE LIMITED | OUR REAGENTS | YOUR RESEARCH |