






# Brucella Antibody IgG GENLISA™ ELISA

**REF** : KBD130

Ver 1.0

**IVD**

Enzyme Immunoassay for Qualitative and Semi-Quantitative Determination of Brucella Antibody IgG in human serum and plasma.

<b>IVD</b>	For In-vitro Diagnostic Use	<b>REF</b>	Catalog Number
	Store At	<b>LOT</b>	Batch Code
	Manufactured By		Biological Risk
	Expiry Date		Consult Operating Instructions

For *In vitro* Diagnostic use only. Purchase does not include or carry the right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of KRISHGEN Pudgala LLP is strictly prohibited.

**REF** KBD130

 96 tests

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**Introduction:**

Brucellosis, also known as “undulant fever”, “Mediterranean fever” or “Malta fever”, is Gram-negative coccobacilli, nonflagellated, non-spore-forming and is a zoonotic infection which is almost invariably transmitted by direct or indirect contact with infected animals or their products (untreated milk and milk products). Of the three species causing human infection, *B. melitensis* is the most pathogenic followed by *B. suis* and *B. abortus*. The incubation period of brucellosis is usually one to three weeks, but sometimes may be several months. The illness may be mild and self-limiting or severe. The disease is accompanied by continued, intermittent, or irregular fever such as malaise, anorexia and prostration and which, in the absence of specific treatment, may persist for weeks or months, also headache, weight loss, and generalized aching and fatigue can be observed. Urogenital symptoms may dominate the clinical presentation in some patients. The outbreaks are seen in many parts of the world especially in the Mediterranean countries of Europe, North and East Africa, the Middle East, South and Central Asia and Central and South America and yet it is often unrecognized and frequently goes unreported.

**Intended Use:**

The Brucella Antibody IgG GENLISA™ ELISA is intended for the qualitative and semi-quantitative determination of IgG class antibodies in human serum and plasma.

**Principle:**

Brucella Antibody IgG GENLISA™ ELISA is an indirect enzyme linked immunosorbent assay for qualitative and semi-quantitative determination of IgG antibody present in the human serum and plasma. Brucella Antigen is pre-coated onto microwells. Samples, Controls are pipetted into microwells and Brucella IgG antibody present in sample binds to the antigen coated on the wells. Enzyme Conjugate antibody is pipetted and incubated to form an immune complex. After washing microwells in order to remove any non-specific binding, the substrate solution is added to microwells and color develops proportionally to the amount of Brucella IgG present in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

**Materials Provided:**

1. Microtiter Coated Plate (96 wells) – 1 no
2. Negative Control – 2 ml
3. Positive Control – 2 ml
4. Cut-Off Control – 2 ml
5. Enzyme Conjugate – 20 ml
6. (20x) Wash Buffer – 25 ml
7. Sample Diluent – 100 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. Graph paper or software for data analysis
6. Timer
7. Absorbent Paper

**Handling/Storage:**

1. Store main kit components at recommended storage temperature indicated on the component label.
2. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.
3. 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. Refer to the MSDS online for details.
2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.



**Specimen Collection and Handling:**

**Serum-** Coagulate at room temperature for 10 - 20 minutes; centrifuge for 20 minutes at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.

**Plasma-** Use EDTA or citrate plasma as an anticoagulant, mix for 10 - 20 minutes; centrifuge for 15 minutes at 2000-3000 rpm. Remove the supernatant carefully. If precipitation appears, recentrifuge.

**Sample Dilution:**

To make 1:100 Dilution, dilute 10 ul Sample + 1 ml Sample Diluent. Mix well.

**Reagent Preparation:**

1. (1x) Wash Buffer Dilution: To make (1x) Wash Buffer, add 25ml of (20x) Wash Buffer to 475ml of DI water. This is the working solution.
2. Allow all components to reach RT (Room Temperature) prior to use in the assay.

**Test Procedure:**

1. All reagents should be allowed to reach room temperature before use.
2. Add **100ul Controls, Diluted Sample** in appropriate wells.
3. Seal the plate and Incubate at 37°C for 60 minutes.
4. Aspirate and wash plate 5 times with **(1x) Wash Buffer** 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. All the washes should be performed similarly.
5. Add **100 ul of Enzyme Conjugate** to each well except blank well. Incubate at 37°C for 30 minutes.
6. Repeat the Wash Step as mentioned in step 4.
7. Add **100 ul of TMB Substrate** into each well.
8. Incubate at 37°C for 10 minutes.
9. Add **100 ul of Stop Solution**. Read result with an ELISA reader at 450 nm within 15 minutes of stopping the reaction.

**Criteria of Validation:**

<b>Blank</b>	O.D < 0.100
<b>Negative Control</b>	O.D < 0.200
<b>Cut-Off Control (CO)</b>	O.D between 0.350 – 0.850
<b>Positive Control</b>	O.D between 0.650 – 3.000

**Calculation:**

Determine the Mean Absorbance (net of Blank) for each set of duplicate Controls and Samples.

$$O.D_{\text{Mean Cut-off Control (CO)}} = O.D_{\text{Cut-Off Control 1}} + O.D_{\text{Cut-Off Control 2}} / 2.$$

**Interpretation:**

<b>Positive</b>	$O.D_{\text{Mean Sample}} > 1.1 \times CO$
<b>Grey - Zone</b>	$0.9 \times CO \leq O.D_{\text{Mean Sample}} \leq 1.1 \times CO$
<b>Negative</b>	$O.D_{\text{Mean Sample}} < 0.9 \times CO$

**Results:**

$$\frac{O.D_{\text{Mean Sample}} \times 10}{CO} = \text{Krishgen Units} = U$$

**Interpretation of Results:**

<b>Cut-Off Value</b>	10 U
<b>Grey – Zone</b>	9 – 11 U
<b>Negative Value</b>	< 9 U
<b>Positive Value</b>	> 11 U

**Reference Values:**

It is recommended that each laboratory establishes its own normal and pathological reference ranges, as usually done for other diagnostic parameters, too.

**Limitations of Method:**

Any clinical diagnosis should not be based on the results of in-vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

**Performance Characteristics:**

**Sensitivity:**

The sensitivity is the probability of the assay of scoring positive in the presence of the specific analyte. The sensitivity is 100 %.

**Specificity:**

The antigens used in this kit are specific to Brucella Antibody.

**Precision:**

Intra-Assay:

Sample	Mean O.D	Intra-Assay CV%	N
1	0.54	5.42	20
2	1.29	3.67	20
3	1.62	2.71	20

Inter-Assay:

Sample	Mean O.D	Inter-Assay CV%	N
1	1.26	7.48	40
2	1.53	7.16	40
3	1.94	4.78	40

**Safety Precautions:**

- **This kit is For In-vitro Diagnostic 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 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 Pudgala LLP does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the product; against defects in products or components not manufactured by Krishgen Pudgala LLP, or against damages resulting from such non-Krishgen Pudgala LLP made products or components. Krishgen Pudgala LLP 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 product to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Pudgala LLP.

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Krishgen Pudgala LLP, 2022.

**THANK YOU FOR USING KRISHGEN PRODUCT!**



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**Regulatory Status:**

CE Marked	Europe
FDA registered	USA
CDSCO registered	India

## SCHEMATIC ASSAY PROCEDURE

1	All reagents should be allowed to reach room temperature before use.
2	Add <b>100 ul Control, Diluted Sample</b> in appropriate wells.
3	Seal the plate and <b>Incubate at 37°C for 60 minutes.</b>
4	Aspirate and wash plate 5 times with (1x) Wash Buffer 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. All the washes should be performed similarly.
5	Add <b>100 ul of Enzyme Conjugate</b> to each well except blank well.
6	<b>Incubate at 37°C for 30 minutes.</b>
7	Repeat the Wash Step as mentioned in step 4.
8	Add <b>100 ul of TMB Substrate</b> into each well.
9	<b>Incubate at 37°C for 10 minutes.</b>
10	Add <b>100 ul of Stop Solution.</b> Read result with an ELISA reader at 450 nm within 15 minutes stopping the reaction.

**SYMBOLS KEY**

	Coated Microtiter Plate (8 x 12 wells)
	Control
	Enzyme Conjugate
	Sample Diluent
	(20x) Wash Buffer
	TMB Substrate
	Stop Solution
	Consult Instructions for Use
	Catalog Number
	Expiration Date
	Storage Temperature