GENLISA® Avian Psittacine Beak and Feather Disease (PBFD / BFD / Circovirus) Antibody ELISA

REF : KLA009

Ver 1.0

RUO

Enzyme Immunoassay for the Qualitative Determination of Avian Psittacine Beak and Feather Disease (PBFD / BFD / Circovirus) Antibody in serum, plasma and same-species 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 **KLA009** **Σ 96 tests**

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Introduction:

The GENLISA® ELISA kits use monoclonal antibodies and recombinant antigens for consistent and reliable results.

Intended Use:

The GENLISA® Avian Psittacine Beak and Feather Disease (PBFD / BFD / Circovirus) Antibody ELISA is used as an analytical tool for qualitative determination in serum and plasma.

Principle:

The method employs a competitive enzyme-linked immunosorbent assay (ELISA) technique to assay the level of Avian Psittacine Beak and Feather Disease (PBFD / BFD / Circovirus) Antibody in samples. Standards or Samples competes with the Anti-Psittacine BFDV Antibody:HRP Conjugate Solution, to form a complex with the Recombinant Beak and feather disease virus (BFDV) Cap protein Antigen coated microtiter well. Wells are washed to remove the excess conjugate. 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 Avian Psittacine Beak and Feather Disease (PBFD / BFD / Circovirus) Antibody in the samples is inversely proportional to the yellow color developed (absorbance) in the wells.

Materials Provided:

Part	Description	Qty
Recombinant BFDV Cap protein Antigen Coated Microtiter Plate	96 well polystyrene microplate (12 strips of 8 wells) coated with Recombinant Beak and feather disease virus (BFDV) Cap protein Antigen Coated Microtiter Plate.	12x8 wells
Anti-Psittacine BFDV Antibody Positive control	Anti-Psittacine Beak and Feather Disease Virus (BFDV) Virus (Antibody in a buffered protein base with preservative 0.02% Methylisothiazolinone and 0.02% bromonitrodioxane – (lyophilized, concentrated).	2 vials
Anti-Psittacine BFDV Antibody:HRP Conjugate, concentrated	Anti-Psittacine Beak and Feather Disease Virus (BFDV) Antibody conjugated to Horseradish Peroxidase, Concentrated.	2 x 35 ul
(1X) Control Diluent	Buffer with protein stabilizer and preservatives 0.02% Methylisothiazolinone and 0.02% bromonitrodioxane.	12 ml
(1X) Sample Diluent	Buffer with protein stabilizer and preservatives 0.02% Methylisothiazolinone and 0.02% bromonitrodioxane	2 x 50 ml
(1X) Detection Diluent	Buffer with protein stabilizer and preservatives 0.02% Methylisothiazolinone and 0.02% bromonitrodioxane .	10 ml
(20X) Wash Buffer	20-fold concentrated solution of buffered surfactant with preservative thiomersal < 0.01%. May turn yellow over time.	25 ml
TMB Substrate	Stabilized TMB Chromogen.	12 ml
Stop Solution	0.73M Phosphoric Acid.	12 ml
Instruction Manual		1 no

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. Timer.
6. Absorbent Paper.

Handling/Storage:

1. It is advisable to aliquot and store the HRP Conjugate concentrated at -20°C upon receipt. Rest of the kit components should be stored at 2-8°C. Immediately discard any excess working reagents after running your assay.
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 Veterinary Use Only.



Sample Preparation and Storage:

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Plasma can be used, too. Lipaemic, hemolytic or contaminated samples should not be run. Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at -20°C.

Preparation Before Use:

Allow samples to reach room temperature prior to assay. Take care to agitate patient samples gently in order to ensure homogeneity.

Test Sample preparation - Serum and plasma samples have to be diluted 1:1000 (v/v), e.g. for 1:1000 (1 ul sample + 999 ul sample diluent) prior to assay. The samples may be kept at 2-8°C for up to three days. Long-term storage requires the samples to be kept at -20°C.

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); dilute 25 ml of 20X Wash Buffer in 475 ml of DI water.
4. **Preparation of Working Anti-Psittacine Beak and Feather Disease Virus (BFDV) Antibody Positive Control –** Reconstitute the lyophilized positive control in 1000ul Control diluent. Dilute 100ul of Reconstituted Positive control to 400ul of Control Diluent to prepare working positive control. Use the working positive control for the experiment.
5. **Control Diluent is used as the Negative control for the kit.**
6. **Working Anti-Psittacine Beak and Feather Disease Virus (BFDV) Antibody:HRP Conjugate, concentrated – Refer to the Reagent Preparation sheet enclosed with the kit (note each Reagent Preparation Sheet is specific for the Lot No of the kit).**

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 protein. High Dose Hook Effect is due to excess of protein present in the sample. To avoid the same, dilute the samples to be assayed with a compatible diluent.

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 total Avian Psittacine Beak and Feather Disease (PBFD / BFD / Circovirus) Antibody.
4. It is recommended that all Controls and Samples be assayed in duplicates.
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 Controls and Samples be run in duplicates or triplicates.

2. Add **100 ul** of **working Positive Control**, **Negative Control and diluted Samples** into the respective wells.

3. Add **100 ul of working Anti-Psittacine Beak and Feather Disease Virus (BFDV) Antibody:HRP Solution** to all wells. Mix thoroughly and Cover with adhesive membrane and Incubate at **37°C for 90 minutes.**

4. Aspirate and wash plate 4 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.

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

6. Incubate the plate at **Room Temperature for 30 minutes** in dark. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.

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

8. Read the absorbance at 450 nm with a microplate reader.

Validity of the test:

The test is valid if the following conditions are met:
Under normal experimental conditions, the absorbance value of the Negative Control should be ≥1.0, and the absorbance value of the Positive Control should be ≤75% of the absorbance value of the Negative Control.

Interpretation of Results:

$$PI\% \text{ (Percentage Inhibition)} = [1 - (Abs_S / Abs_{NC})] \times 100\%$$

% Inhibition	Interpretation
< 50%	Negative
50 - 65 %	Equivocal
>= 65%	Positive

Note:
Differences in baseline absorbance and % inhibition may occur among different psittacine species. The assay has been evaluated using representative species; however, samples with results in the inconclusive range should be retested or interpreted with caution and confirmed using an alternative method where appropriate.

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.

Safety Precautions:

- **This kit is For Veterinary 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/v) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from **animal body fluids** or organs used in the preparation of this kit were tested and found negative for viral antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all 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 (Good Laboratory Practice) should be applied with all general and individual regulations to the use of this kit.

Typical Example of a Work List

Well #	Contents	Absorbance at 450nm	Mean Absorbance	Results
1A 2A	Blank Blank			
1B 2B	Control Control			
1C 2C	Sample Sample			
1D 2D	Sample Sample			
1E 2E	Sample Sample			
1F 2F	Sample Sample			
1G 2G	Sample Sample			
1H 2H	Sample Sample			
3A 4A	Sample Sample			
3B 4B	Sample Sample			

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SYMBOLS KEY

MTP	Recombinant BFDV Cap protein Antigen Coated Microtiter Plate (12 x 8 wells)
PC	Anti-Psittacine BFDV Antibody Positive control, lyophilized
HRP CONJ	Anti-Psittacine BFDV Antibody:HRP Conjugate, concentrated
1X SAMP DIL	(1X) Sample Diluent
1X CNTRL DIL	(1X) Control Diluent
1X DETN DIL	(1X) Detection Diluent
20X WASH BUF	(20X) Wash Buffer
SUB TMB	TMB Substrate
SOLN STOP	Stop Solution
	Consult Instructions for Use
REF	Catalog Number
	Expiry Date
	Storage Temperature