Bovine Immunoglobulin A (IgA) ELISA
Cat No: K04-0109

Principle:
The Bovine Immunoglobulin A (IgA) ELISA is competitive enzyme-linked immunosorbent assay (ELISA) to assay the level of IgA in samples. Standards or Samples competes with the antibody coated microtiter well to form a complex with the HRP labelled antigen. 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 Bovine Immunoglobulin A (IgA) in the samples is inversely proportional to the yellow color developed (absorbance) in the wells.

Intended Use:
This Kit is used to assay the level of Bovine Immunoglobulin A (IgA) in Bovine serum and plasma samples and other biological samples. The Kit is For Laboratory / Research Use Only.

Materials provided in the Kit:
1. Bovine IgA Antibody Coated Microtiter Plate (96 wells) - 1 no
2. Lyophilized Bovine IgA Standard (Concentrated, 200 ng/ml) - 2 vials
3. HRP labelled Antigen - 60 ul
4. Sample Diluent - 20 ml
5. Antigen Dilution Buffer - 10 ml
6. (25X) Wash Buffer - 30 ml
7. TMB Substrate - 10 ml
8. Stop Solution - 10 ml
9. Instruction Manual

Materials to be provided by the End-User:
1. Microplate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes to measure volumes ranging from 50 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.

Storage Information:
1. All reagents should be stored at 2ºC to 8ºC For long term storage, store the biotin antibody and standards (preferably aliquoted) at -20ºC. Avoid multiple freeze-thaws as it leads to loss of activity of the components.
2. All the reagents and wash solutions are stable until the expiration date of the kit.
3. 20 minutes prior before use, bring all components to room temperature (18-25ºC). Store all the components of the kit at its appropriate storage condition after use.
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. Refer to the MSDS online for details.
2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all samples in accordance with NCCLS regulations.

Specimen Collection and Handling:

1. **Serum-** Place whole blood sample at room temperature for 2 hours or put it at 2-8°C overnight and centrifugation for 20 minutes at approximately 1000×g, Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and non-endotoxin.
2. **Plasma-** Collect plasma using EDTA-Na2 or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2-8°C within 30 minutes of collection. Collect the supernatant and carry out the assay immediately. Avoid hemolysis, high cholesterol samples.
3. **Tissue Homogenates-** As hemolysis blood has relation to the assay results, it is necessary to remove residual blood by washing tissue with pre-coating PBS buffer (0.01M, pH=7.4). Mince tissue after weighing it and get it homogenized in PBS (the volume depends on the weight of the tissue Normal 9 ml PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitors are recommended to add into the PBS) with a glass homogenizer or ice. To further break the cells, you can sonicate the suspension with an ultrasonic cell disruptor or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 minutes at 5000xg to get the supernatant. The total protein concentration was determined by BCA kit and the total protein concentration of each pore sample should not exceed 0.3 mg,
4. **Cell Culture Supernatant-** Centrifuge supernatant for 20 minutes at 1000xg at 2-8°C to remove insoluble impurity and cell debris. Collect the clear supernatant and carry out the assay immediately.
5. **Cell Culture Lysate-** Commercial RIPA kits are recommended to follow the instructions provided. Generally 0.5 ml RIPA lysis buffer would be appropriate to 2x10(6) cells, DNA must be removed. The total protein concentration was determined by BCA kit and the total protein concentration of each pore sample should not exceed 0.3 mg.
6. **Other Biological Fluids-** Centrifuge samples for 20 minutes are 1000xg at 2-8°C. Collect supernatant and carry out the assay immediately.

Note: Samples to be used within 5 days can be stored at 2-8°C, besides that, samples must be stored at -20°C (assay ≤1 month) or -80°C(assay≤2 months) to avoid loss of bioactivity and contamination. Avoid multiple freeze-thaw cycles. The hemolytic samples are not suitable for this assay.

Sample Dilution

The user should estimate the concentration of target protein in the test sample, and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit. Dilute the sample with the provided diluent, and several trials may be necessary. The test sample must be well mixed with the diluent. And also standard curves and sample should be making in pre-experiment. If samples with very high concentrations, dilute samples with PBS first and then dilute the samples with Sample Dilution.

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

Bring all reagents to Room Temperature for 20 minutes prior to use.

1. **Wash Buffer**:
   If crystals have formed in the concentrate, you can warm it with 40°C water bath (Heating temperature should not exceed 50°C) and mix it gently until the crystals have completely been dissolved. The solution should be cooled to room temperature before use.
   Dilute 30 ml (25X) Wash Buffer into 720 ml Deionized or Distilled Water to obtain 750 ml (1X) Wash Buffer.

2. **Standards:**
   Add 0.5 ml Sample Diluent to the Bovine IgA Standard vial provided in the kit. Keep the vial at room temperature for 10 minutes and mix thoroughly before making further dilutions. Prepare the **Standards** by serially diluting the standard stock solution as per the below table. The sample diluent is the Standard No. 1 or blank for this competitive assay.

Standard Concentration	Standard Vial	Dilution Particulars
200 ng/ml	Standard No.8	Reconstitute with 1ml Sample Diluent
100 ng/ml	Standard No.7	300 ul Standard No.8 + 300 ul Sample Diluent
50 ng/ml	Standard No.6	300 ul Standard No.7 + 300 ul Sample Diluent
25 ng/ml	Standard No.5	300 ul Standard No.6 + 300 ul Sample Diluent
12.5 ng/ml	Standard No.4	300 ul Standard No.5 + 300 ul Sample Diluent
6.25 ng/ml	Standard No.3	300 ul Standard No.4 + 300 ul Sample Diluent
3.125 ng/ml	Standard No.2	300 ul Standard No.3 + 300 ul Sample Diluent
0 ng/ml	Standard No.1	300 ul Sample Diluent only

Note: It is best to use the prepared Standards within 2 hours of preparation.

3. **Preparation of HRP labelled Antibody Working Solution:**
   Prepare within 30 minutes before your assay.
   Dilute the HRP labelled Antigen with Antigen Dilution Buffer at 1:100 and mix thoroughly. (for example Add 1 ul of HRP labelled Antigen to 99 ul of Antigen 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 Bovine Immunoglobulin A (IgA). High Dose Hook Effect is due to excess of antibody for very high concentrations of Bovine Immunoglobulin A (IgA) present in the sample. High Dose Hook effect is most likely encountered from samples early in the purification process. If Hook Effect is possible, the samples to be assayed should be diluted with a compatible diluent. Thus if the Bovine Immunoglobulin A (IgA) concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect.
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 Bovine Immunoglobulin A (IgA).
4. It is recommended that all Standards 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 poor 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:

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

1) Add **100 ul** of **prepared Standards** or **Samples** to respective wells.

2) Add **100 ul HRP labelled Antigen Working Solution** to each well. Mix well.

3) Cover with the plate and Incubate for 30 minutes at 37°C.

4) 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.

5) Pipette **90 ul TMB Substrate** into each well.

6) Incubate the plate at **37°C** for **10 minutes**. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color

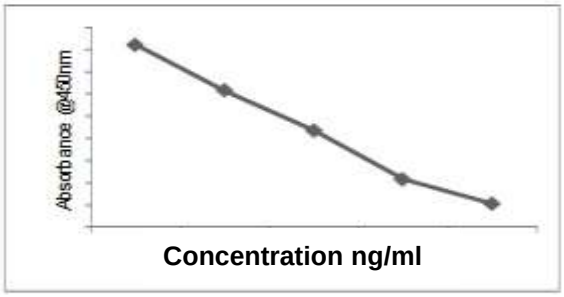
7) Pipette **50 ul** of **Stop Solution** to all wells. The wells should turn from blue to yellow in color.

8) 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. Use the Net Absorbance (Absorbance of Standard/Sample - Absorbance of Blank) to calculate the Mean Absorbances. Using standard graph paper, plot the average value (absorbance 450nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points. To determine the unknown concentrations, find the unknown's Mean Absorbance value on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the concentration. If samples were diluted, multiply by the appropriate dilution factor.

Software which is able to generate a cubic spline curve-fit, 4PL or a polynomial regression to the 2nd order is best recommended for automated results.

Typical Graph



Precautions:
Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this set.

Performance Characteristics:
Please note that this validation is performed in our laboratory and will not necessarily be duplicated in your laboratory. This data has been generated to enable the user to get a preview of the assay and the characteristics of the kit and is generic in nature. We recommend that the user performs at the minimum; the spike and recovery assay and the dilutional linearity assay to assure quality results. For a more comprehensive validation, the user may run the protocols as suggested by us herein below to develop the parameters for quality control to be used with the kit.

Sensitivity:
Limit Of Detection: It is defined as the lowest detectable concentration corresponding to a signal of Mean of '0' standard plus 2* SD. 10 replicates of '0' standards were evaluated and the LOD was found to **1.875ng/ml**.

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

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

Matrix	Recovery Range (%)	Average (%)
Serum(n=5)	86-100	92
EDTA Plasma(n=5)	85-103	95
Heparin Plasma(n=5)	89-99	93
Serum(n=5)	86-100	92

Assay Range:
3.125 ng/ml - 200ng/ml

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

Linearity:
The linearity of the kit was assayed by testing samples spiked with appropriate concentration of IGA and their serial dilutions. The results were demonstrated by percentage of calculated concentration to the expectation.

Sample	1:2	1:4	1:8
serum (n=5)	88-95%	95-105%	86-101%
EDTA plasma (n=5)	82-89%	85-95%	86-98%
heparin plasma (n=5)	90-99%	82-96%	80-89%

LIMITED WARRANTY

KinesisDx 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 KinesisDx, or against damages resulting from such non-KinesisDx made products or components. KinesisDx 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 KinesisDx.

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

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

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ASSAY PROCEDURE

1	Bring all reagents to room temperature before use.		
2	Pipette Standards 1 - 8 Samples	100 ul	100 ul
3	Pipette HRP labelled Antigen	100 ul	100 ul
4	Incubate	30 minutes (37ºC)	
5	Wash 1X Wash Buffer	Decant, 4 x 300 ul	
6	TMB Substrate	90 ul	90 ul
7	Incubate in the dark	10 minutes (37ºC)	
8	Pipette Stop Solution	50 ul	50 ul
9	Measure 450 within 15 mins		