Goat Immunoglobulin A (IgA) ELISA

Enzyme Immunoassay for the estimation of Goat Immunoglobulin A (IgA)

REF : KINE5063

Ver 1.0

For Veterinary Use Only

Σ 1 x 96 wells

Store at 2 - 8 ºC

KINETIC BIOTECH FZCO

Building A1,
Dubai Digital Park,
Dubai Silicon Oasis, Dubai,
UAE
Tel: +44-186-5522721
Email: info@kineticbiotech.ae

Ste#619,
606 S Hill Street,
Los Angeles, CA 90014.
USA

Introduction:

The Kinetic Biotech ELISA kits use monoclonal antibodies and recombinant antigens for consistent and reliable results.

Intended Use:

The Goat Immunoglobulin A (IgA) ELISA is used as an analytical tool for qualitative determination in serum and plasma.

Principle:

This ELISA is a sandwich immunoassay. Antibodies are coated on 96 well plates. The antigen protein present in sample and standard respectively bind to the coated wells. The wells are washed and an antibody:HRP Conjugate is added which binds to the bound complex in the well. Washing is performed to remove any unbound material. TMB substrate is added and the enzyme reaction is stopped by dispensing of stop solution into the wells. The optical density (OD) of the solution at 450 nm is directly proportional to the amount of antigen protein present in the standard or samples.

Materials Provided:

1. Coated Microtiter Plate, 1 x 96 wells
2. Control Vial, 1 vial
3. HRP Conjugate, 1 vial
4. Wash Buffer, 1 vial
5. TMB Substrate, 1 vial
6. Stop Solution , 1 vial
7. Diluent (refer Kit)
8. 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. 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.

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.

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 Immunoglobulin A.
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. A standard curve is required for each assay. All steps must be performed at 37°C.

2. Pipette out **50 ul** of **Controls** or **Samples** in each well.

3. Pipette out 10 ul of Controls and 40 ul of Diluent in each well.

4. Add **100 ul** of **HRP detection conjugate** into the respective wells.

5. Incubate at 37°C for 60 minutes.

6. 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.

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

8. Incubate the plate at 37°C for 15-20 minutes in dark. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.

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

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

Interpretation of Results:

Determine the Mean Absorbance for each set of Control(s) and Samples. Calculate the S/Co Ratio as under - The S/Co ratio is calculated by dividing the optical density (OD) or signal strength of the test sample by the optical density value of the Control.

$$S/Co = \frac{\text{Sample OD405}}{\text{Positive Contorl OD450}}$$

S/Co	**< 0.9**	**Sample is Non-Reactive / Negative**
S/Co	**>0.9 - <1.0**	**Sample is Equivocal.** *Please retest or interpret with Clinical Symptoms*
S/Co	**> 1.0**	**Sample is Reactive / 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.

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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