IgA (Horse) ELISA Kit

Catalog Number KA2035

96 assay

Version: 05

Intended for research use only

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Introduction

Intended Use

The IgA (Horse) ELISA Kit is a highly sensitive two-site enzyme-linked immunoassay (ELISA) for measuring IgA in horse biological samples. If the ELISA is to be used outside the intended use, the user may need to optimize for said use.

Principle of the Assay

The principle of the double antibody sandwich ELISA is represented in Figure 1. In this assay the IgA present in samples reacts with the anti-IgA antibodies, which have been adsorbed to the surface of polystyrene microtitre wells. After the removal of unbound proteins by washing, anti-IgA antibodies conjugated with horseradish peroxidase (HRP) are added. These enzyme-labeled antibodies form complexes with the previously bound IgA. Following another washing step, the enzyme bound to the immunosorbent is assayed by the addition of a chromogenic substrate, 3,3',5,5'-tetramethylbenzidine (TMB). The quantity of bound enzyme varies directly with the concentration of IgA in the sample tested; thus, the absorbance, at 450 nm, is a measure of the concentration of IgA in the test sample. The quantity of IgA in the test sample can be interpolated from the standard curve constructed from the standards, and corrected for sample dilution.

Figure 1.

Anti-IgA Antibody Bound To Solid Phase

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Standards and Samples Added

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IgA * Anti-IgA Complexes Formed

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Unbound Sample Proteins Removed

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Anti-IgA-HRP Conjugate Added

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Anti-IgA-HRP * IgA * Anti-IgA Complexes Formed

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Unbound Anti-IgA-HRP Removed

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Chromogenic Substrate Added

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Determine Bound Enzyme Activity

General Information

Materials Supplied

List of component

Component	Description	Amount
ELISA Micro Plate, antibody coated	One plate of 12 removable 8 well strips, antibody coated. Ready to use as supplied.	96 (8x12) wells
Enzyme Conjugated Detection Antibody	One vial of 100X Horseradish Peroxidase Conjugated antibody in a stabilizing buffer. Dilute 1/100 immediately prior to use.	150 µL
Calibrator	One vial of calibrator. Refer to Certificate of Analysis (CoA).	1 vial
Diluent Concentrate	One bottle of 5X diluent buffer. Dilute 1/5 to make 1X working solution.	50 mL
Wash Solution Concentrate	One bottle of 20X wash solution. Dilute 1/20 to make 1X working solution.	50 mL
Chromogen Substrate Solution	One bottle of 3,3',5,5'- tetramethylbenzidine (TMB) and hydrogen peroxide in citric acid buffer at pH 3.3. Ready to use as supplied.	12 mL
STOP Solution	One bottle of 0.3 M sulfuric acid. WARNING: Avoid Contact with Skin. Ready to use as supplied.	12 mL

Storage Instruction

The expiration date for the kit and its components is stated on the box label. All components should be stable up to the expiration date if stored and used per this kit protocol insert.

Component	Storage	Stability
ELISA Micro Plate, antibody coated	4-8°C, in sealed foil bag with desiccant.	With proper storage the plate strips are stable until the expiration date.
Enzyme Conjugated Detection Antibody	4-8°C in the dark.	The working conjugate solution should be diluted immediately prior to use. The 100X conjugate is stable until the expiration date.
Calibrator	4-8°C for lyophilized calibrator. Aliquoted and frozen if reconstituted. Avoid multiple freeze-thaw cycles.	The working standard solutions should be prepared immediately prior to use.
Diluent Concentrate	4-8°C for both 1X working solution and 5X concentrate.	The 1X working solution is stable for at least one week from the date of preparation. The 5X concentrate is stable until the expiration date.

Wash Solution Concentrate	4-8°C for both 1X working solution and 20X concentrate.	The 1X working solution is stable for at least one week from the date of preparation. The 20X concentrate is stable until the expiration date.
Chromogen Substrate Solution	4-8°C in the dark.	Protect from light. The Substrate Solution is stable until the expiration date.
STOP Solution	4-8°C.	The Stop Solution is stable until the expiration date.

Materials Required but Not Supplied

- ✓ Precision pipette (2 µL to 100 µL) for making and dispensing dilutions
- ✓ Test tubes
- ✓ Squirt bottler or Microtitre washer/aspirator
- ✓ Distilled or Deionized H_2O
- ✓ Microtitre Plate reader
- ✓ Assorted glassware for the preparation of reagents and buffer solutions
- ✓ Centrifuge for sample collection
- ✓ Anticoagulant for plasma collection
- ✓ Timer

Precautions for Use

Please read this protocol completely before using this product.
For Research Use Only, Not for Diagnostic Purposes. Not for Human and Animal Consumption. For In Vitro Laboratory Use Only.

- ✓ Limitation of the procedure

  Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the information contained in the package insert instructions and with adherence to good laboratory practice. Factors that might affect the performance of the assay include instrument function, cleanliness of glassware, quality of distilled or deionized water, and accuracy of reagent and sample pipetting, washing technique, incubation time or temperature. Do not mix or substitute reagents with those from other lots or sources.

Assay Protocol

Reagent Preparation

Bring all reagents to room temperature (16°C to 25°C) before use.

- ✓ Diluent Concentrate

  The Diluent solution supplied is a 5X Concentrate and must be diluted 1/5 with distilled or deionized water (1 part buffer concentrate, 4 parts dH_2O).

- ✓ Wash Solution Concentrate.

  The Wash Solution supplied is a 20X concentrate and must be diluted 1/20 with distilled or deionized water (1 part buffer concentrate, 19 parts dH_2O). Crystal formation in the concentrate may occur when storage temperatures are low. Warming of the concentrate to 30-35°C before dilution can dissolve crystals.

- ✓ Enzyme-Antibody Conjugate

  Calculate the required amount of working conjugate solution for each microtiter plate test strip by adding 10 µL Enzyme-Antibody Conjugate to 990 µL of 1X Diluent for each test strip to be used for testing. Dilute immediately before use and protect from light. Mix uniformly, but gently. Avoid foaming.

- ✓ Pre-coated ELISA Micro Plate

  Ready to use as supplied. Unseal foil pouch and remove plate from pouch. Remove all strips and wells that will not be used in the assay and place back in pouch and re-seal along with desiccant.

- ✓ Horse IgA Calibrator

  Prepare according to the lot specific Certificate of Analysis.

Sample Preparation

- Specimen Collection and Handing

  All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions when handling and disposing.

  If blood samples are clotted, grossly hemolyzed, lipemic, or the integrity of the sample is of concern, make a note and interpret results with caution.

  The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

  - Serum samples

    Blood should be collected by venipuncture. The serum should be separated from the cells after clot formation by centrifugation. Remove serum and assay immediately or aliquot and store samples at -80°C (preferably) or -20°C. Avoid repeated freeze-thaw cycles.

  - Plasma samples

    Blood should be collected into a container with an anticoagulant and then centrifuged. Assay immediately or aliquot and store samples at -80°C (preferably) or -20°C. Avoid repeated freeze-thaw cycles.

- Urine samples

  Collect mid-stream using sterile or clean urine collector. Centrifuge to remove cell debris. Assay immediately or aliquot and store samples at -80°C (preferably) or -20°C. Avoid repeated freeze-thaw cycles.
- Known interfering substances

  Azide and thimerosal at concentrations higher than 0.1% inhibits the enzyme reaction.

- Dilution of Samples

  The assay requires that each test sample be diluted before use. All samples should be assayed in duplicate each time the assay is performed. The recommended dilutions are only suggestions. Dilutions should be based on the expected concentration of the unknown sample such that the diluted sample falls within the dynamic range of the standard curve. If unsure of sample level, a serial dilution with one or two representative samples before running the entire plate is highly recommended.
  - Serum samples

    Recommended starting dilution is 1/10,000. To prepare a 1/10,000 dilution of a sample, transfer 5 µL of sample to 495 µL of 1X diluent. This gives you a 1/100 dilution. Next, dilute the 1/100 by transferring 5 µL into 495 µL of 1X diluent. This gives you a 1/10,000 dilution. Mix thoroughly each stage.
  - Plasma samples

    Recommended starting dilution is 1/10,000. To prepare a 1/10,000 dilution of a sample, transfer 5 µL of sample to 495 µL of 1X diluent. This gives you a 1/100 dilution. Next, dilute the 1/100 by transferring 5 µL into 495 µL of 1X diluent. This gives you a 1/10,000 dilution. Mix thoroughly each stage.

Assay Procedure

1. All samples and standards should be assayed in duplicates.
2. The Standards and the test sample(s) should be loaded into the ELISA wells as quickly as possible to avoid a shift in OD readings. Using a multichannel pipette would reduce this occurrence.

   Pipette 100 µL of

   Standard 0 (0.0 ng/mL) in duplicate

   Standard 1 (15.63 ng/mL) in duplicate

   Standard 2 (31.25 ng/mL) in duplicate

   Standard 3 (62.5 ng/mL) in duplicate

   Standard 4 (125 ng/mL) in duplicate

   Standard 5 (250 ng/mL) in duplicate

   Standard 6 (500 ng/mL) in duplicate
3. Pipette 100 µL of sample (in duplicate) into pre designated wells.
4. Incubate the Microtiter Plate at room temperature for thirty (30 ± 2) minutes. Keep plate covered and level during incubation.

5. Following incubation, aspirate the contents of the wells.
6. Completely fill each well with appropriately diluted Wash Solution and aspirate. Repeat three times, for a total of four washes. If washing manually: completely fill wells with wash buffer, invert the plate and pour/shake out the contents in a waste container. Follow this by sharply striking the wells on absorbent paper to remove residual buffer. Repeat 3 times for a total of four washes.
7. Pipette 100 µL of appropriately diluted Enzyme-Antibody Conjugate to each well. Incubate at room temperature for thirty (30 ± 2) minutes. Keep plate covered in the dark and level during incubation.
8. Wash and blot the wells as described in Steps 5/6.
9. Pipette 100 µL of TMB Substrate Solution into each well.
10. Incubate in the dark at room temperature for precisely ten (10) minutes.
11. After ten minutes, add 100 µL of Stop Solution to each well.
12. Determine the absorbance (450 nm) of the contents of each well within 30 minutes. Calibrate the plate reader to manufacturer's specifications.

Data Analysis

Calculation of Results

1. Subtract the average background value (Average absorbance reading of Standard zero) from the test values for each sample.
2. Average the duplicate readings for each standard and use the results to construct a Standard Curve. Construct the standard curve by reducing the data using computer software capable of generating a four parameter logistic curve fit. A second order polynomial (quadratic) or other curve fits may also be used; however, they will be a less precise fit of the data.
3. Interpolate test sample values from standard curve. Correct for sera dilution factor to arrive at the IgG concentration in original samples.

Resources

Plate Layout

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11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H