IgG (Horse) ELISA Kit

Catalog Number KA2036

96 assay

Version: 04

Intended for research use only

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Introduction

Intended Use

The IgG (Horse) ELISA Kit is a highly sensitive two-site enzyme-linked immunoassay (ELISA) for measuring of IgG in horses' biological samples. For research use only.

Principle of the Assay

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

Figure 1.

Anti-IgG Antibody Bound To Solid Phase

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Standards and Samples Added

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IgG * Anti-IgG Complexes Formed

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Unbound Sample Proteins Removed

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Anti-IgG-HRP Conjugate Added

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Anti-IgG-HRP * IgG * Anti-IgG Complexes Formed

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Unbound Anti-IgG-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	Amount
Diluent Concentrate (Running Buffer): One bottle containing a 5X concentrated diluent running buffer.	50 mL
Wash Solution Concentrate: One bottle containing a 20X concentrated wash solution.	50 mL
Enzyme-Antibody Conjugate 100X: One vial containing affinity purified anti-Horse IgG antibody conjugated with horseradish peroxidase in stabilizing buffer.	150 µL
Chromogenic-Substrate Solution: One vial containing 3,3',5,5'-tetramethybenzidine (TMB) and hydrogen peroxide in citric acid buffer at pH 3.3.	12 mL
Stop Solution: One vial containing 0.3 M sulfuric acid. WARNING: Avoid contact with skin	12 mL
Anti-Horse IgG ELISA Micro plate: Twelve removable eight (8) well strips in well holder frame. Each well is coated with affinity purified anti-Horse IgG.	96 (8x12) wells
Horse IgG Calibrator: One vial containing lyophilized Horse IgG Calibrator.	1 vial

Storage Instruction

The expiration date for the package is stated on the box label.

- ✓ Diluent
  The 5X Diluent Concentrate is stable until the expiration date. The 1X working solution is stable for at least one week from the date of preparation. Both solutions should be stored at 4-8°C.
- ✓ Wash Solution
  The 20X Wash Solution Concentrate is stable until the expiration date. The 1X working solution is stable for at least one week from the date of preparation. Both solutions can be stored at room temperature (16-25°C) or at 4-8°C.
- ✓ Enzyme-Antibody Conjugate
  Undiluted horseradish peroxidase anti-IgG conjugate should be stored at 4-8°C and diluted immediately prior to use. The working conjugate solution is stable for up to 1 hour when stored in the dark.
- ✓ Chromogen-Substrate Solution
  The Substrate Solution should be stored at 4-8°C and is stable until the expiration date.
- ✓ Stop Solution
  The Stop Solution should be stored at 4-8°C and is stable until the expiration date.
- ✓ Anti-Horse IgG ELISA Micro Plate
  Anti-Horse IgG coated wells are stable until the expiration date and should be stored at 4-8°C in the sealed foil pouch with a desiccant pack.

✓ Horse IgG Calibrator

The lyophilized Horse IgG Calibrator should be stored at 4°C or frozen until reconstituted. The reconstituted calibrator should be aliquoted out and stored frozen (Avoid multi freeze-thaw cycles). The working standard solutions should be prepared immediately prior to use.

Materials Required but Not Supplied

- ✓ Precision pipette (2 µL to 200 µL) for making and dispensing dilutions
- ✓ Test tubes
- ✓ Microplate washer/aspirator
- ✓ Distilled or Deionized H_2O
- ✓ Microtitre plate reader
- ✓ Assorted glassware for the preparation of reagents and buffer solutions
- ✓ Timer

Precautions for Use

✓ Precausion

For any sample that might contain pathogens, care must be taken to prevent contact with open wounds.

✓ Additives and Preservatives

No additives or preservatives are necessary to maintain the integrity of the specimen. Avoid azide contamination.

✓ Known interfering substances

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

✓ Limitation of the procedure

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

Assay Protocol

Reagent Preparation

- ✓ 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 is not uncommon 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. Mix uniformly, but gently. Avoid foaming.
- ✓ Chromogen-Substrate Solution

  Ready to use as supplied.
- ✓ Stop Solution

  Ready to use as supplied.
- ✓ Anti-Horse IgG ELISA Micro Plate

  Ready to use as supplied. Unseal Microtiter 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.
- ✓ Horse IgG Calibrator

  Add 1.0 mL of distilled or deionized water to the Horse IgG Calibrator and mix gently until dissolved. The calibrator is now at a concentration of 4.94 mg/mL (the reconstituted calibrator should be aliquoted and frozen if future use is intended). Horse IgG standards need to be prepared immediately prior to use (See chart below). Mix well between each step. Avoid foaming.

Standard	ng/mL	Volume added to 1X Diluent	Volume of 1X Diluent
A	49400	5 µL of Horse IgG Calibrator	495 µL
7	400	6 µL of standard A	735 µL
6	200	300 µL standard 7	300 µL
5	100	300 µL standard 6	300 µL
4	50	300 µL standard 5	300 µL
3	25	300 µL standard 4	300 µL
2	12.5	300 µL standard 3	300 µL
1	6.25	300 µL standard 2	300 µL
0	0		600 µL

Sample Preparation

✓ Specimen Collection and Handling

Blood should be collected by venipuncture. The serum should be separated from the cells after clot formation by centrifugation. For plasma samples, blood should be collected into a container with an anticoagulant and then centrifuged. Care should be taken to minimize hemolysis, excessive hemolysis can impact your results. Assay immediately or aliquot and store samples at -20°C. Avoid repeated freeze thaw cycles.

✓ Dilution of Samples

The assay for quantification of IgG in samples requires that each test sample be diluted before use. For a single step determination a dilution of 1/200,000 is appropriate for most serum/plasma samples. For absolute quantification, samples that yield results outside the range of the calibration curve, a lesser or greater dilution might be required. If unsure of sample level, a serial dilution with one or two representative samples before running the entire plate is highly recommended.

- To prepare a 1/200,000 dilution of sample, transfer 2 µL of sample to 1,998 µL of 1X Diluent. This gives you a 1/1,000 dilution. Next, dilute the 1/1,000 sample by transferring 2 µL to 398 µL of 1X Diluent. You now have a 1/200,000 dilution of your sample. Mix thoroughly at each stage.

Assay Procedure

1. Bring all reagents to room temperature before use.
2. Pipette 100 µL of
   - Standard 0 (0.0 ng/mL) in duplicate
   - Standard 1 (6.25 ng/mL) in duplicate
   - Standard 2 (12.5 ng/mL) in duplicate
   - Standard 3 (25 ng/mL) in duplicate
   - Standard 4 (50 ng/mL) in duplicate
   - Standard 5 (100 ng/mL) in duplicate
   - Standard 6 (200 ng/mL) in duplicate
   - Standard 7 (400 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 twenty (20 ± 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. Calibrate the plate reader to manufacturer's specifications.

✓ Stability of the final reaction mixture

The absorbance of the final reaction mixture can be measured up to 2 hours after the addition of the Stop Solution. However, good laboratory practice dictates that the measurement be made as soon as possible.

Data Analysis

Calculation of Results

1. Subtract the average background value from the test values for each sample.
2. Using the results observed for the standards construct a standard curve. The appropriate curve fit is that of a four-parameter logistics curve. A second order polynomial (quadratic) or other curve fits may also be used.
3. Interpolate test sample values from the standard curve. Correct for sera dilution factor to arrive at the IgG concentration in original samples.

✓ Indication of instability

If the test is performing correctly, the results observed with the standard solutions should be within 20% of the expected values.

Resources

Plate Layout

12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H