



GUINEA PIG IgG ELISA Assay Kit

Immunoperoxidase Assay for Determination of IgG in Guinea Pig Samples

GPG91-K01

DIRECTIONS FOR USE

For Research Use Only, NOT for Diagnostic Purposes

Please Read this Package Insert Completely Before Using This Product

INTENDED USE

The Guinea Pig IgG ELISA Assay Kit is a highly sensitive two-site enzyme linked immunoassay (ELISA) for measuring IgG in biological samples of Guinea Pigs.

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 microtitre 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.

REAGENTS

(Quantities sufficient for 96 determinations)

- 1. DILUENT CONCENTRATE (Running Buffer)**
One bottle containing 50 ml of a 5X concentrated diluent running buffer.
- 2. WASH SOLUTION CONCENTRATE**
One bottle containing 50 ml of a 20X concentrated wash solution.
- 3. ENZYME-ANTIBODY CONJUGATE 100X**
One vial containing 150 µL of affinity purified anti-Guinea Pig IgG antibody conjugated with horseradish peroxidase in a stabilizing buffer.
- 4. CHROMOGEN-SUBSTRATE SOLUTION**
One vial containing 12 mL of 3,3',5,5'-tetramethylbenzidine (TMB) and hydrogen peroxide in citric acid buffer at pH 3.3.
- 5. STOP SOLUTION**
One vial containing 12 ml 0.3 M sulfuric acid.
- 6. ANTI-GUINEA PIG IgG ELISA MICRO PLATE**
Twelve removable eight (8) well micro well strips in well holder frame. Each well is coated with affinity purified anti-Guinea Pig IgG.
- 7. GUINEA PIG IgG STANDARDS**
One vial containing Guinea Pig IgG calibrator.

WARNING: Avoid contact with skin.

FOR RESEARCH USE ONLY

REAGENT PREPARATION

- 1. DILUENT CONCENTRATE**
The Diluent Solution supplied is a 5X Concentrate and must be diluted 1/5 (1 part buffer concentrate, 4 parts dH2O) with distilled or deionized water.

2. 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 part dH₂O). 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.

3. ENZYME-ANTIBODY CONJUGATE

Calculate the required amount of working conjugate solution for each microtitre 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.

4. CHROMOGEN-SUBSTRATE SOLUTION

Ready to use as supplied.

5. STOP SOLUTION

Ready to use as supplied.

6. ANTI-GUINEA PIG 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. Take clear plastic tape and cover tops of strips to avoid particulates from contaminating wells.

7. GUINEA PIG IgG STANDARDS

The Guinea Pig IgG Calibrator should be aliquoted out and stored frozen. It is at a concentration of 4.616 mg/ml and needs to be diluted in 1X diluent immediately prior to use for each run (see chart below). Mix well between each step. Avoid foaming.

Standard	ng/ml	Volume added to 1x Diluent	Volume of 1x Diluent
A	46160	5 µl Guinea Pig IgG Calibrator	495 µl
7	400	5 µl standard A	572 µl
6	200	250 µl standard 7	250 µl
5	100	250 µl standard 6	250 µl
4	50	250 µl standard 5	250 µl
3	25	250 µl standard 4	250 µl
2	12.5	250 µl standard 3	250 µl
1	6.25	250 µl standard 2	250 µl
0			500 µl

STORAGE AND STABILITY

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

1. 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.

2. 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.

3. 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.

4. CHROMOGEN-SUBSTRATE SOLUTION

The Substrate Solution should be stored at 4-8°C and is stable until the expiration date.

5. STOP SOLUTION

The Stop Solution should be stored at 4-8°C and is stable until the expiration date.

6. ANTI-GUINEA PIG IgG ELISA MICRO PLATE

Anti-Guinea Pig IgG coated wells are stable until the expiration date, and should be stored at 4-8°C in sealed foil pouch with desiccant pack.

7. GUINEA PIG IgG STANDARDS

Aliquot Guinea Pig IgG calibrator and store them frozen, avoid repeated freeze-thaw cycles. For storage longer than 14 days, keep frozen until the expiration date. Storage of less than 14 days can be 4°C. The working standard solutions should be prepared immediately prior to use and are stable for up to 8 hours.

SPECIMEN COLLECTION AND HANDLING

Blood should be collected by venipuncture and the serum separated from the cells, after clot formation, by centrifugation. Care should be taken to minimize hemolysis, excessive hemolysis can impact your results. Specimens may be shipped at room temperature and then stored refrigerated at 2-8°C if testing is to take place within one week after collection. If testing is to take place later than one week, specimens should be stored at -20°C. Avoid repeated freeze-thaw cycles.

1. Precautions

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

2. Additives and Preservatives

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

3. Known interfering substances

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

MATERIAL PROVIDED

See "REAGENTS"

MATERIALS REQUIRED BUT NOT PROVIDED

- Precision pipette (2 μ L to 200 μ L) for making and dispensing dilutions
- Test tubes
- Microtitre washer/aspirator
- Distilled or Deionized H₂O
- Microtitre Plate reader
- Assorted glassware for the preparation of reagents and buffer solutions
- Timer

ASSAY PROTOCOL

DILUTION OF SERUM SAMPLES

The assay for quantification of IgG in serum requires that each test sample be diluted before use. For a single step determination a dilution of serum at 1/100,000 is appropriate for most samples. **For absolute quantification, samples that yield results outside the range of the standard curve, a lesser or greater dilution might be required. If unsure of sample level**

1. To prepare a 1/100,000 dilution of sample, transfer 2 μ L of sample to 1,998 μ L of 1X diluent. This gives you a 1/1,000 dilution. Next, dilute the 1/1,000 samples by transferring 10 μ L, to 990 μ L of 1X diluent. You now have a 1/100,000 dilution of your sample. Mix thoroughly at each stage.

PROCEDURE

1. **Bring all reagents to room temperature before use.**

2. Pipette 100 μ L 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 μ L of sample (in duplicate) into pre designated wells.

4. Incubate the micro titer plate at room temperature for twenty (20 \pm 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 then 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 μ L of appropriately diluted Enzyme-Antibody Conjugate to each well. Incubate at room temperature for twenty (20 \pm 2) minutes. Keep plate covered and level during incubation.

8. Wash and blot the wells as described in Steps 5/6.

9. Pipette 100 μ L 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 μ L 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.

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 standard curve. Correct for sera dilution factor to arrive at the IgG concentration in original sample.

LIMITATION OF THE PROCEDURE

1. 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.

2. 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.

EAGLE BIOSCIENCES, INC.
20A NW BLVD, SUITE 112 NASHUA, NH 03063
P: 617-419-2019 F: 617-419-1110
WWW.EAGLEBIO.COM — INFO@EAGLEBIO.COM



EAGLE
BIOSCIENCES

LIMITED WARRANTY

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

Eagle Biosciences makes no warranties, either expressed or implied, except as provided herein, including without limitation thereof, warranties as to marketability, merchantability, fitness for a particular purpose or use, or noninfringement of any intellectual property rights. In no event shall the company be liable for any indirect, incidental, or consequential damages of any nature, or losses or expenses resulting from any defective product or the use of any product. Product(s) may not be resold, modified, or altered for resale without prior written approval from Eagle Biosciences, Inc.

For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.

THANK YOU FOR USING EAGLE BIOSCIENCES!