PLG (Pig) ELISA Kit

Catalog Number KA1959

96 assays

Version: 01

Intended for research use only

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Introduction and Background

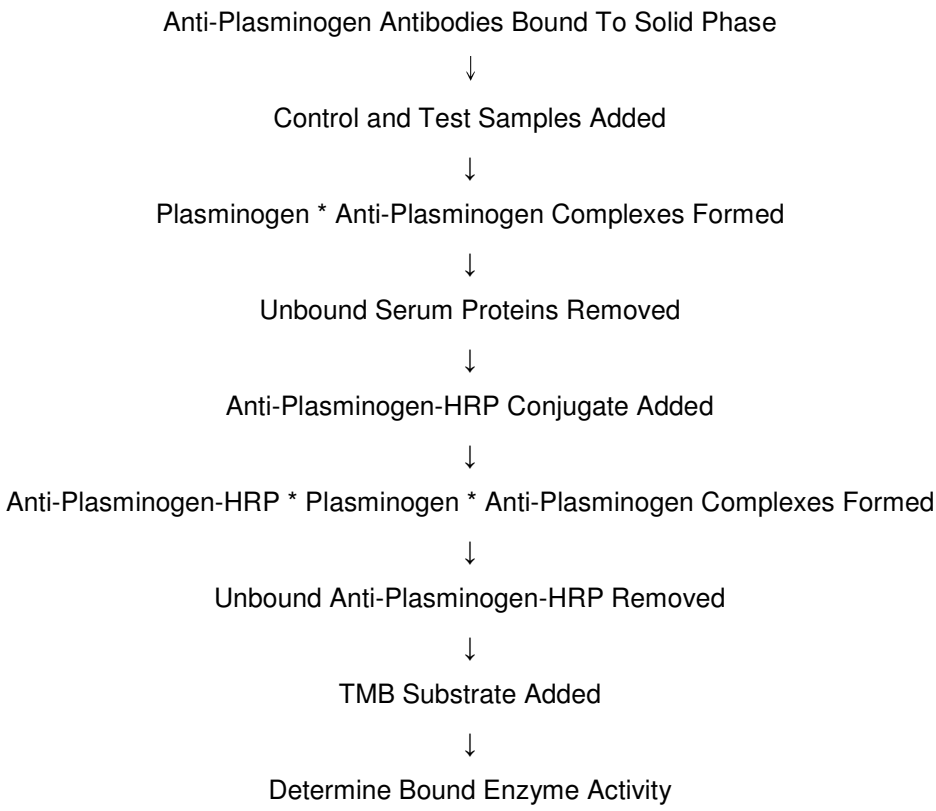
A. Introduction

Plasminogen (PMG) is a glycoprotein produced by the liver. It is the precursor for plasmin, which targets fibrin in the process of dissolution of fibrin blood clots. Plasminogen is present in plasma and most extravascular fluids. The important role of plasminogen in the fibrinolytic system makes it an interesting marker for various diseases.

B. Test principle

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

Figure 1.



C. Intended use

The PLG (Pig) ELISA Kit is a highly sensitive two-site enzyme-linked immunoassay (ELISA) for the quantitative determination of Plasminogen in pig biological fluids. For research use only.

Material and Method

A. List of component

1. Diluent Concentrate: One bottle containing 50 mL of a 5X concentrated phosphate buffered saline (PBS) solution containing 0.25% Tween, protein stabilizer and 0.25% Proclin 300 as a preservative.
2. Wash Solution Concentrate: One bottle containing 50 mL of a 20X concentrated PBS solution with 1% Tween.
3. Enzyme-Antibody Conjugate Concentrate: One vial containing 200 µL of a 100X concentrated affinity-purified anti-pig Plasminogen antibody conjugated with HRP in a stabilizing buffer.
4. TMB Substrate Solution: One vial containing 12 mL of TMB and hydrogen peroxide in citric acid buffer at pH 3.3.
5. Stop Solution: One vial containing 12 mL of 0.3 M sulfuric acid. WARNING: Avoid contact with skin.
6. Microtiter Plate: Twelve removable eight-well strips in well holder frame. Wells are coated with affinity-purified anti-pig Plasminogen.
7. Pig Plasminogen Calibrator: One vial containing a lyophilized Pig Plasminogen Calibrator.

B. Additional required materials but not provided

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

C. Precautions

1. Read the instructions carefully before beginning the assay.
2. This kit is for research use only.
3. Great care has been taken to ensure the quality and reliability of this product. However, it is possible that in certain cases, unusual results may be obtained due to high levels of interfering factors.
4. Preservatives: Diluent contains 0.25% Proclin 300 as a preservative.
5. No additives or preservatives are necessary to maintain the integrity of the specimen. Avoid azide contamination.
6. Azide and thimerosal at concentrations higher than 0.1% inhibit the enzyme reaction.
7. Other precautions:
   - Do not interchange kit components from different lots.
   - Do not use kit components beyond the expiration date.
   - Protect reagents from direct sunlight.
   - Do not pipette by mouth.

- Do not eat, drink, smoke or apply cosmetics where reagents are used.
- Avoid all contact with the reagents by using gloves.
- Stop solution contains diluted sulfuric acid. Irritation to eyes and skin is possible. Flush with water after contact.

D. Reagent preparation

1. Diluent Concentrate

   The Diluent solution supplied is a 5X concentrate and must be diluted 1:5 with distilled or de-ionized water.

2. Wash Solution Concentrate

   The Wash Solution supplied is a 20X concentrate and must be diluted 1:20 with distilled or de-ionized water. Crystal formation in the concentrate is not uncommon when storage temperatures are low. Warming of the concentrate to 30-35℃ before dilution can dissolve crystals.

3. Enzyme-Antibody Conjugate Concentrate

   The Enzyme-Antibody Conjugate supplied is a 100X concentrate and must be diluted 1:100. The required amount of working conjugate solution for each microtiter plate is prepared by adding 100 µL Enzyme-Antibody Conjugate to 9.9 mL of 1X Diluent. Mix uniformly, but gently. Avoid foaming.

4. TMB Substrate Solution

   Ready to use as supplied.

5. Stop Solution

   Ready to use as supplied.

6. Microtiter Plate

   Ready to use as supplied.

7. Pig Plasminogen Calibrator

   Add 1.0 mL of distilled or de-ionized water to the lyophilized Pig Plasminogen Calibrator and mix gently until dissolved. The calibrator is now at a concentration of 19.06 µg/mL (reconstituted calibrator should be aliquoted and frozen if future use is intended). Pig Plasminogen calibrators need to be prepared immediately prior to use (see the following chart). Mix well between each step. Avoid foaming.

Calibrator	Concentration (ng/mL)	Calibrator Volume added to 1X Diluent	Volume of 1X Diluent
6	200	10.5 µL Plasminogen Calibrator	990 µL
5	100	500 µL Calibrator 1	500 µL
4	50	500 µL Calibrator 2	500 µL
3	25	500 µL Calibrator 3	500 µL
2	12.5	500 µL Calibrator 4	500 µL
1	6.25	500 µL Calibrator 5	500 µL
0	3.125	500 µL Calibrator 6	500 µL

E. Storage and stability

1. Complete Kit

   The expiration date for the kit is stated on the outer label. The recommended storage temperature is 4℃.

   Note: See long term storage recommendations below for the Pig Plasminogen Calibrator.

2. 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℃.

3. 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 (RT, 16-25℃) or at 4℃.

4. Enzyme-Antibody Conjugate

   Undiluted horseradish peroxidase anti-Plasminogen conjugate should be stored at 4℃ and diluted immediately prior to use. The working conjugate solution is stable for one day.

5. TMB Substrate Solution

   The TMB Substrate Solution should be stored at 4℃ and is stable until the expiration date.

6. Stop Solution

   The Stop Solution should be stored at 4℃ and is stable until the expiration date.

7. Microtiter Plate

   Anti-pig Plasminogen coated wells are stable until the expiration date and should be stored at 4℃ in the sealed foil pouch with a desiccant pack.

8. Pig Plasminogen Calibrator

   The lyophilized Pig Plasminogen Calibrator should be stored at 4℃ or frozen until reconstituted. The reconstituted calibrator should be aliquoted and stored frozen (avoid multiple freeze-thaw cycles). The working calibrator solutions should be prepared immediately prior to use and are stable for one day.

F. Indications of instability

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

G. 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 (RT) and then stored refrigerated at 4℃ 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℃. Avoid repeated freezing/thawing. For any sample that might contain pathogens, care must be taken to prevent contact with open wounds. No additives or preservatives are necessary to maintain the integrity of the specimen. Avoid azide contamination.

H. Assay protocol

- **Dilution of Samples**

Due to the high-sensitive nature of the assay, each test sample should be diluted before use for a normal assay. A 1:10,000 dilution is appropriate for most serum samples. For absolute quantification of samples that yield results outside the range of the calibration curve, a lesser or greater dilution might be required.

To prepare a 1:10,000 dilution of sample, transfer 5 µL of sample to 495 µL of 1X Diluent. This gives you a 1:100 dilution. Next, dilute the 1:100 sample by transferring 10 µL to 990 µL of 1X Diluent. You now have a 1:10,000 dilution of your sample. Mix thoroughly at each step.

- **Procedure**

Bring all reagents to RT before use.

1. Add 100 µL of 1X Diluent to each of the wells in A1 & A2. These will serve for an evaluation of the background associated with the assay.
2. Pipette 100 µL of
   Calibrator 1 (200 ng/mL) into wells B1 & B2
   Calibrator 2 (100 ng/mL) into wells C1 & C2
   Calibrator 3 (50 ng/mL) into wells D1 & D2
   Calibrator 4 (25 ng/mL) into wells E1 & E2
   Calibrator 5 (12.5 ng/mL) into wells F1 & F2
   Calibrator 6 (6.25 ng/mL) into wells G1 & G2
   Calibrator 7 (3.125 ng/mL) into wells H1 & H2
3. Pipette 100 µL of diluted serum sample (test sample 1) into wells A3 & A4. The next sample goes in wells B3 & B4, the next in C3 & C4 and so on.
4. Incubate the Microtiter Plate at 22℃ (RT) for thirty (30 ± 2) minutes. Keep plate 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. Finally, invert the plate on absorbent paper (paper towel) and blot the excess fluid from the wells.
7. Pipette 100 µL of appropriately diluted Enzyme-Antibody Conjugate to each well. Incubate at 22℃ (RT) for thirty (30 ± 2) minutes.
8. Wash and blot the wells as described in Steps 5 and 6.

9. Pipette 100 µL of TMB Substrate Solution into each well.
10. Incubate at RT for precisely ten (10) minutes.
11. After ten (10) minutes, add 100 µL of Stop Solution to each well.
12. Determine the absorbance at 450 nm of the contents of each well. Zero the plate reader to air. The absorbance of the final reaction mixture can be measured up to two hours after the addition of the Stop Solution. However, good laboratory practice dictates that the measurement be made as soon as possible.

I. Results

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

J. Quality control

In accord with good laboratory practice, the assays for specific Plasminogen require meticulous quality control. Each laboratory should use routine quality control procedures to establish inter- and intra-assay precision and performance characteristics.

K. 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 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 de-ionized water, washing thoroughly and accuracy of reagent and sample pipetting.