HP (Rabbit) ELISA Kit

Catalog Number KA1930

96 assays

Version: 01

Intended for research use only

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Introduction and Background

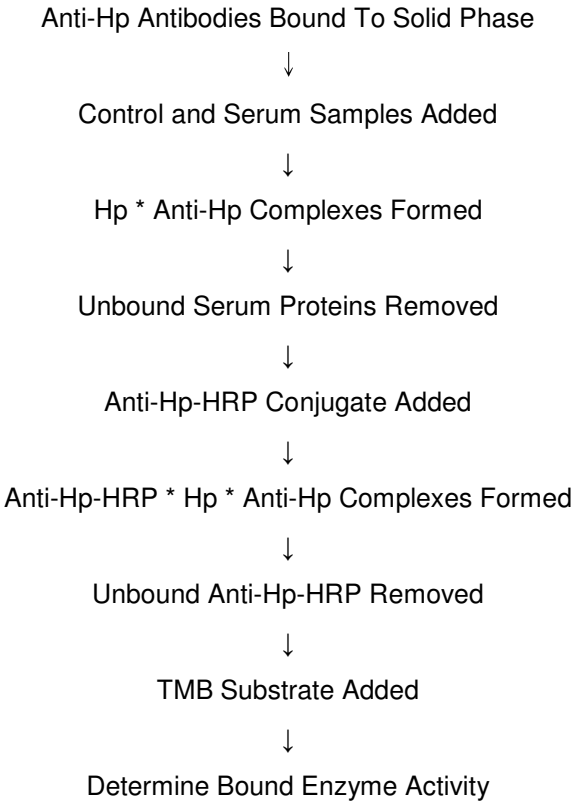
A. Introduction

Acute phase proteins are plasma proteins, which increase in concentration following infection, inflammation or trauma. The first acute phase protein to be recognized was discovered in humans by Tillet and Frances in 1930. Haptoglobin (Hp) is a heterogeneous plasma protein mostly synthesized by the liver. The haptoglobin monomer consists of two heavy chains, beta chains (40 kDa), and two light chains, alpha chains, alpha 1 (9 kDa) and alpha 2 (16 kDa) that are linked by disulfide bonds. The three major haptoglobin types are; Hp1-1, which is monomeric (98 kDa), Hp1-2 is polymeric at about 200 kDa, and Hp2-2 at about 400 kDa. The haptoglobin level in serum rises quickly following acute tissue damage within 24 to 48 hours and also falls very rapidly once the stimulus is removed. In fact, haptoglobin levels are decreased in hemolytic anemia. Haptoglobin has a high affinity for hemoglobin (Hb) and its function appears to be to prevent loss of hemoglobin in urine, which would lead to loss of iron. Investigations over the past few years have shown that quantification of haptoglobin in plasma or serum can provide valuable information in the detection, prognosis, and monitoring of disease not only in humans, but in companion animals and farm herds as well.

B. Test principle

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

Figure 1.



C. Intended use

The HP (Rabbit) ELISA Kit is a highly sensitive two-site enzyme-linked immunoassay (ELISA) for the quantitative determination of Haptoglobin in rabbit serum or plasma. 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-rabbit haptoglobin 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-rabbit haptoglobin.
7. Rabbit Haptoglobin Calibrator: One vial containing a lyophilized Rabbit Haptoglobin Calibrator.
8. Positive Control: One vial containing 50 µL of serum with 0.1% sodium azide. See the Control Certificate for the concentration.

B. Additional required materials but not provided

- ✓ Test tubes
- ✓ Precision pipette (2 µL to 200 µL)
- ✓ 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. Positive Control contains 0.1% sodium azide.
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. Rabbit Haptoglobin Calibrator

   Add 1.0 mL of distilled or de-ionized water to the lyophilized Rabbit Haptoglobin Calibrator and mix gently until dissolved. The calibrator is now at a concentration of 23 µg/mL (the reconstituted calibrator should be aliquoted and frozen if future use is intended). Prepare the Rabbit Haptoglobin Calibrators immediately prior to use according to the table below. Mix well between each step. Avoid foaming.

Calibrator	Concentration (ng/mL)	Calibrator Volume added to 1X Diluent	Volume of 1X Diluent
1	200	5 µL Rabbit Haptoglobin Calibrator	570 µL
2	100	0.3 mL Calibrator 1	0.3 mL
3	50	0.3 mL Calibrator 2	0.3 mL
4	25	0.3 mL Calibrator 3	0.3 mL
5	12.5	0.3 mL Calibrator 4	0.3 mL
6	6.25	0.3 mL Calibrator 5	0.3 mL

8. Positive Control

   The concentration and recommended dilution are provided on the Control Certificate. Before use, briefly centrifuge the Positive Control to allow all of the liquid to collect in the bottom of the vial.

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 Rabbit Haptoglobin Calibrator and Positive Control.**

2. Diluent Concentrate

   The 5X Diluent Concentrate should be stored at 4℃ and is stable until the expiration date.

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

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

8. Rabbit Haptoglobin Calibrator

   The lyophilized Rabbit Haptoglobin 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.

9. Positive Control

   For storage longer than 7 days keep frozen until the expiration date. Storage less than 7 days can be at 4℃. Avoid multiple freeze/thaw cycles.

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. 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 serum sample should be diluted before use for a normal assay. A 1:4,000 dilution is appropriate for most serum or plasma 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 1:4,000 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 390 µL of 1X Diluent. You now have a 1:4,000 dilution of your sample. Mix thoroughly at each stage.

- **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
3. Pipette 100 µL of diluted Positive Control into wells H1 & H2.
4. 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.
5. Incubate the Microtiter Plate at 22℃ (RT) for sixty (60 ± 2) minutes. Keep plate level during incubation.
6. Following incubation, aspirate the contents of the wells.
7. 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 diluted Wash Solution, 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 Wash Solution. Repeat three times for a total of four washes.
8. Pipette 100 µL of appropriately diluted Enzyme-Antibody Conjugate to each well. Incubate at 22℃ (RT) for twenty (20 ± 2) minutes.

9. Wash and blot the wells as described in Steps 6 and 7.
10. Pipette 100 µL of TMB Substrate Solution into each well.
11. Incubate at RT for precisely ten (10) minutes.
12. After ten minutes, add 100 µL of Stop Solution to each well.
13. 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 haptoglobin concentration in original sample.

J. Quality control

In accord with good laboratory practice, the assays for specific haptoglobin 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.