KAMIYA BIOMEDICAL COMPANY

Cat Haptoglobin ELISA

For the quantitative determination of haptoglobin in cat serum and plasma.

Cat. No. KT-422

For Research Use Only.

PRODUCT INFORMATION

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PRODUCT
The **K-ASSAY®** Cat Haptoglobin ELISA is an enzyme immunoassay for the quantitative determination of haptoglobin in cat serum and plasma. For research use only.

INTRODUCTION
Haptoglobin is an acute phase protein, the concentrations of which can increase two to ten fold in cat serum as a result of disease. Measurement of haptoglobin provides a convenient marker of inflammation and disease in cats.

PRINCIPLE
The **K-ASSAY®** Cat Haptoglobin ELISA is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay uses affinity purified anti-cat haptoglobin antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated anti-cat haptoglobin antibodies for detection. The test sample is diluted and incubated in the microtiter wells for 45 minutes. The microtiter wells are subsequently washed and HRP conjugate is added and incubated for 45 minutes. This results in haptoglobin molecules being sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-labeled antibodies and TMB Reagent is added and incubated for 20 minutes at room temperature. This results in the development of a blue color. Color development is stopped by the addition of Stop Solution, changing the color to yellow, and optical density is measured spectrophotometrically at 450 nm. The concentration of haptoglobin is proportional to the optical density of the test sample.

COMPONENTS
- Anti-cat haptoglobin antibody coated microtiter plate with 96 wells (provided as 12 detachable strips of 8)
- Enzyme Conjugate Reagent, 11 mL
- Cat Haptoglobin Calibrator (lyophilized)
- Wash Buffer (20X), 50 mL
- Diluent (10X), 25 mL
- TMB Reagent (One-Step), 11 mL
- Stop Solution (1N HCl), 11 mL

MATERIALS REQUIRED BUT NOT PROVIDED
- Precision pipettes and tips
- Distilled or de-ionized water
- Polypropylene or glass tubes
- Vortex mixer
- Absorbent paper or paper towels
- Micro-Plate incubator/shaker mixing speed of ~150 rpm
- Plate reader with an optical density range of 0-4 at 450 nm
- Graph paper (PC graphing software is optional)

GENERAL INSTRUCTIONS
All reagents should be allowed to reach room temperature (18-25℃) before use.

WASH SOLUTION PREPARATION
The wash solution is provided as a 20X stock. Prior to use dilute the contents of the bottle (50 mL) with 950 mL of distilled or de-ionized water.

DILUENT PREPARATION

The diluent is provided as a 10X stock. Prior to use estimate the final volume of diluent required for your assay and dilute one (1) volume of the 10X stock with nine (9) volumes of distilled or de-ionized water.

CALIBRATOR PREPARATION

1. The Cat Haptoglobin Calibrator is provided as a lyophilized stock. Add the volume of distilled or de-ionized water indicated on the reference calibrator vial label and mix gently until dissolved. (**the reconstituted calibrator remains stable for at least 1 day at 4°C but should be aliquoted and frozen at -20°C after reconstitution if use beyond this time is intended).**
2. Label 8 polypropylene or glass tubes as 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39 and 0 ng/mL.
3. Prepare the 25 ng/mL calibrator as described on the reference calibrator vial label.
4. Dispense 300 µL of diluent into the tubes labeled 12.5, 6.25, 3.125, 1.56, 0.78, 0.39 and 0 ng/mL.
5. Pipette 300 µL of the 25 ng/mL haptoglobin calibrator into the tube labeled 12.5 ng/mL and mix. This provides the working 12.5 ng/mL haptoglobin calibrator.
6. Similarly prepare the 6.25, 3.125, 1.56, 0.78 and 0.39 ng/mL calibrators by serial dilution.

SAMPLE PREPARATION

General Note: Haptoglobin is present in normal cat serum at a concentration of ~1 mg/mL. In order to obtain values within the range of the calibration curve, we suggest that samples be diluted 100,000 fold using the following procedure for each sample to be tested:

1. Dispense 998 µL and 497.5 µL of 1X diluent into separate tubes.
2. Pipette and mix 2 µL of the serum/plasma sample into the tube containing 998 µL of diluent. This provides a 500 fold diluted sample.
3. Mix 2.5 µL of the 500 fold diluted sample with the 497.5 µL of diluent in the second tube. This provides a 100,000 fold dilution of the sample.
4. Repeat this procedure for each sample to be tested.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µL of calibrators and diluted samples into the wells (we recommend that samples be tested in duplicate).
3. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 45 minutes.
4. Remove the incubation mixture using either a plate washer or by flicking plate contents into an appropriate Bio-waste container.
5. Wash and empty the microtiter wells 5 times with 1X wash solution. This should preferentially be performed using a plate washer (400 µL/well). If a plate washer is not available use a squirt bottle. The entire wash procedure should be performed as quickly as possible.
6. Strike the wells sharply onto adsorbent paper or paper towels to remove all residual droplets.
7. Add 100 µL of enzyme conjugate reagent into each well.
8. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 45 minutes.
9. Wash as detailed in 4 to 5 above.
10. Strike the wells sharply onto absorbent paper or paper towels to remove residual droplets.
11. Dispense 100 µL of TMB Reagent into each well.
12. Gently mix on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 20 minutes.
13. Stop the reaction by adding 100 µL of Stop Solution to each well.
14. Gently mix. *It is important to make sure that all the blue color changes to yellow.*
15. Read the optical density at 450 nm with a microtiter plate reader *within 5 minutes.*

CALCULATION OF RESULTS

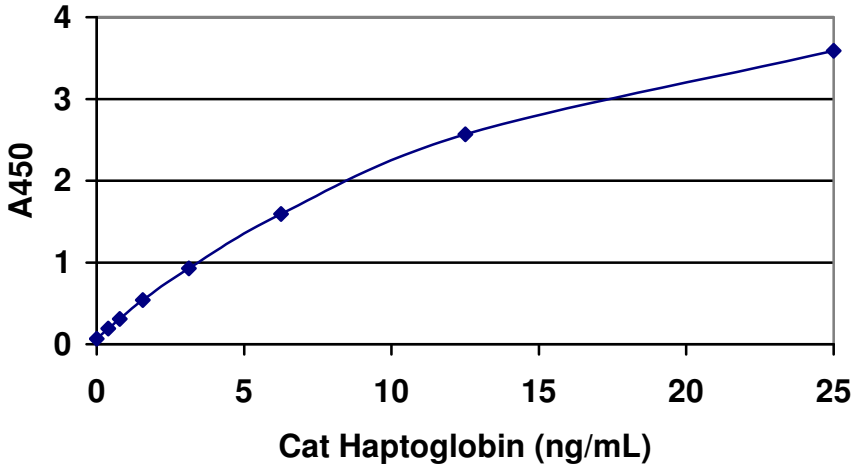
1. Calculate the average absorbance values (A_{450}) for each set of reference calibrators, and samples.
2. Construct a calibration curve by plotting the mean absorbance obtained from each reference calibrator against its concentration in ng/mL on linear graph paper, with absorbance values on the vertical or Y-axis and concentration on the horizontal or X-axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of haptoglobin in ng/mL from the calibration curve.
4. Multiply the derived concentration by the dilution factor to determine the actual concentration of haptoglobin in the serum/plasma sample.
5. PC graphing software may be used for the above steps.
6. If the A_{450} values of samples fall outside the calibration curve when tested at dilution of 100,000, samples should be diluted appropriately and re-tested.

TYPICAL CALIBRATION CURVE

A typical calibration curve with optical density reading at 450 nm on the Y axis against haptoglobin concentration on the X axis is shown below. This curve is for the purpose of illustration only and should not be used to calculate unknowns. Each user should obtain his or her data and calibration curve in each experiment.

Haptoglobin (ng/mL)	Absorbance (450 nm)
25	3.590
12.5	2.567
6.25	1.592
3.13	0.928
1.56	0.541
0.78	0.310
0.39	0.192
0	0.068

Typical Cat Haptoglobin Calibration Curve



STORAGE

The kit should be stored at 4℃ and the microtiter strips should be kept in a sealed bag with desiccant to minimize exposure to damp air. The kit will remain stable until the expiration date provided that the components are stored as described above.

LIMITATIONS OF THE PROCEDURE

1. Do not use grossly hemolyzed samples. Serum hemoglobin concentrations of 0.1 mg/mL have no effect but concentrations of 1 mg/mL cause an approximate 20% decrease in apparent haptoglobin levels.
2. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of and in accordance with the instructions.
3. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

FOR RESEARCH USE ONLY

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