KAMIYA BIOMEDICAL COMPANY

Alpaca Haptoglobin ELISA

For the quantitative determination of haptoglobin in alpaca serum, plasma and milk

Cat. No. KT-788

For Research Use Only.

PRODUCT INFORMATION

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

INTRODUCTION
Haptoglobin is an acute phase protein that is elevated in the blood of most mammals during infection and inflammation. Studies have reported that serum haptoglobin levels increase in alpacas after exposure to lipopolysaccharide, which induces an acute phase response. This kit can be used to measure haptoglobin in serum, plasma and milk.

PRINCIPLE
The **K-*ASSAY*®** Alpaca Haptoglobin ELISA is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay uses affinity purified anti-alpaca haptoglobin antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated anti-alpaca haptoglobin antibodies for detection. Serum, plasma or milk samples are diluted and incubated alongside calibrators in the microtiter wells for 45 minutes. The microtiter wells are subsequently washed. HRP conjugate is added and incubated for 45 minutes. Haptoglobin molecules, if present, are thereby sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-conjugate. 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. Optical density is measured spectrophotometrically at 450 nm. The concentration of haptoglobin is proportional to the optical density of the test sample and is derived from a calibration curve.

COMPONENTS
- Anti-camelid haptoglobin antibody coated microtiter plate with 96 wells (provided as 12 detachable strips of 8)
- HRP Conjugate, 11 mL
- Reference calibrator (lyophilized)
- 20X Wash Buffer, 50 mL
- 10X Diluent, 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
- Microplate incubator/shaker with mixing speed of ~150 rpm
- Plate reader with an optical density range of 0-4 at 450 nm
- PC graphing software or graph paper

STORAGE
The unopened kit should be stored at 4℃ and the microtiter strips should be kept in a sealed bag with desiccant. Test kits will remain stable until the expiration date provided that the components are stored as described above.

GENERAL INSTRUCTIONS
All reagents should be allowed to reach room temperature (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. Reconstitute the lyophilized calibrator stock vial as described on the vial label. The reconstituted stock is stable for one day at 4°C but should be aliquoted and frozen at -20°C or lower if future use is intended.
2. Label 7 polypropylene or glass tubes: 200, 100, 50, 25, 12.5, 6.25 and 0 ng/mL.
3. Prepare a 200 ng/mL working haptoglobin calibrator as detailed on the calibrator vial label by mixing the indicated volume of diluent and reconstituted stock in the tube labeled 200 ng/mL.
4. Dispense 250 µL of diluent into the tubes labeled 100, 50, 25, 12.5, 6.25 and 0 ng/mL.
5. Prepare a 100 ng/mL calibrator by diluting and mixing 250 µL of the 200 ng/mL calibrator with 250 µL of diluent in the tube labeled 100 ng/mL. Similarly prepare the 50, 25, 12.5, and 6.25 ng/mL calibrators by serial dilution.

SAMPLE PREPARATION

We found haptoglobin levels of approximately 4 mg/mL in serum from healthy alpacas. In order to obtain values within the range of the calibration curve, we suggest that samples be diluted 16,000 fold using the following procedure for each sample to be tested:

1. Dispense 198 µL and 636 µL of 1X diluent into separate tubes.
2. Pipette and mix 2 µL of the serum/plasma sample into the tube containing 198 µL of 1X diluent. This provides a 100 fold dilution.
3. Mix 4 µL of the 100 fold diluted sample with the 636 µL of 1X diluent in the second tube. This provides a 16,000 fold dilution of the sample.

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 microplate shaker at 100-150 rpm at room temperature (25°C) for 45 minutes.
4. Remove the incubation mixture using a plate washer and wash the wells 5 times with 1X wash solution (400 µL/well).
5. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
6. Add 100 µL of HRP conjugate to each well.
7. Incubate on an orbital microplate shaker at 100-150 rpm at room temperature (25°C) for 45 minutes.
8. Wash as detailed in 4 and 5 above.
9. Dispense 100 µL of TMB Reagent into each well.
10. Gently mix on an orbital microplate shaker at 100-150 rpm at room temperature (25°C) for 20 minutes.
11. Stop the reaction by adding 100 µL of Stop Solution to each well.
12. Gently mix. It is important to make sure that all the blue color changes to yellow.
13. Read the optical density at 450 nm with a microtiter plate reader within 5 minutes.

CALCULATION OF RESULTS

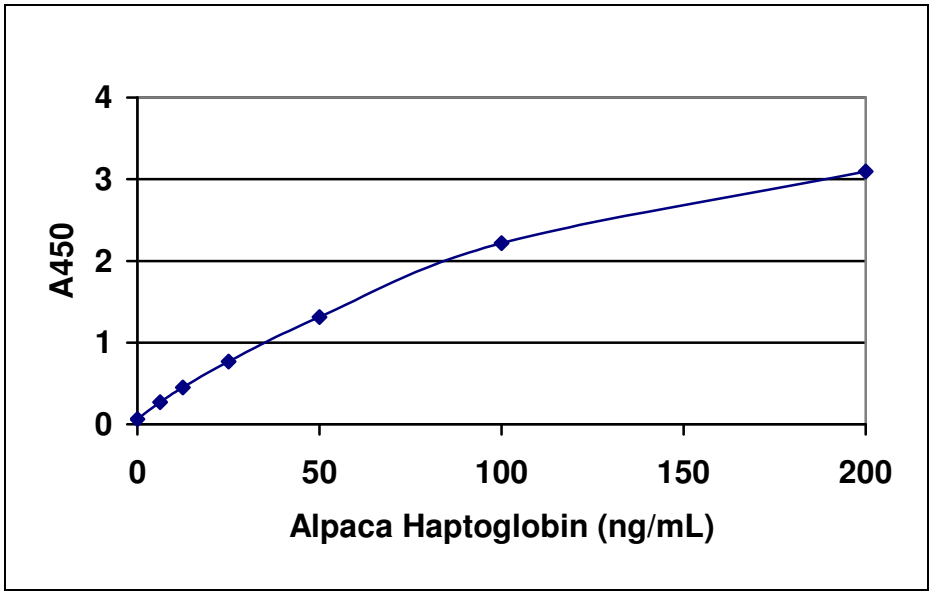
1. Calculate the average absorbance values (A_{450}) for each set of reference calibrators and samples.
2. Construct a calibration curve by plotting the mean absorbance obtained from each reference calibrator against its concentration in ng/mL on linear graph paper, with absorbance values on the vertical or Y-axis and concentrations 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, if available, should be used for the above steps.
6. If the A_{450} values of samples fall outside the range of the calibration curve, samples should be diluted appropriately and re-tested.

TYPICAL CALIBRATION CURVE

A typical calibration curve with optical density readings at 450 nm on the Y-axis against haptoglobin concentrations 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)	A450
200	3.094
100	2.217
50	1.312
25	0.769
12.5	0.449
6.25	0.270
0	0.063



LIMITATIONS OF THE PROCEDURE

1. 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.
2. 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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