Canine / Feline Haptoglobin ELISA Kit

Canine / Feline Haptoglobin ELISA Kit is an enzyme immunoassay kit for the quantification determination Haptoglobin concentration in Canine / Feline serum, plasma and cell culture.

Catalog number: ARG83083

Package: 96 wells

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INTRODUCTION

Haptoglobin (Hp) is an acute phase protein. The plasma concentration of Hp increases rapidly following tissue damage associated with infection and inflammation. Thus Hp levels could be used as a screening test for organic disease, an objective index of disease activity and response to therapy, or as a sign of microbial infection.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for Haptoglobin has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Haptoglobin present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-conjugated antibody specific for Haptoglobin is added to each well and incubate. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Haptoglobin bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm. The concentration of Haptoglobin in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store all other components at 2-8°C. Microtiter wells must be stored at 2-8°C.

Once the foil bag has been opened, care should be taken to close it tightly again.

Use the kit before expiration date.

Component	Quantity	Storage information
Antibody Coated microplate	8 X 12 strips	4°C
50X Standards Buffer	0.2 ml (100 µg/ml)	4°C
10X Standard / Sample Dilution Buffer	30 ml	4°C
50X Wash Buffer	30 ml	4°C
Assay Buffer	12 ml (ready to use)	4°C
HRP-Streptavidin buffer	12 ml (ready to use)	4°C
TMB substrate	12 ml (ready to use)	4°C (protect from light)
STOP solution	12 ml (ready to use)	4°C

Note:

Additional Standard 0 for sample dilution is available upon request.

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 450 nm
- Deionized or distilled water
- Pipettes and pipette tips
- Microtiter plate washer (recommended)

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times. Opened reagents must be stored at 2-8 °C. Microtiter wells must be stored at 2-8°C. Once the foil bag has been opened, care should be taken to close it tightly again. Opened kits retain activity for 4 weeks if stored as described above.
- Briefly spin down the buffer before use.
- If crystals are observed in the 50X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Bring all reagents and required number of strips to room temperature prior to use.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All reagents must be mixed without foaming before use.
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- Once the test has been started, all steps should be completed without interruption.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum: Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Patients receiving anticoagulant therapy may require increased clotting time. Remove serum and assay immediately. The samples can be stored at 2-8 °C up to 7 days or aliquot and store samples at ≤ -20 °C or up to one month. And the sample at -20°C should be frozen only once. Avoid repeated freeze-thaw cycles.

Plasma: Collect plasma using EDTA, citrate or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately. The samples can be stored at 2-8 °C up to 7 days or aliquot and store samples at ≤-20 °C or lower for up to one month. And the sample at -20°C should be frozen only once. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X Standard / Sample Dilution Buffer: Dilute 10X Standard / Sample Dilution Buffer into distilled water to yield 1X Standard / Sample Dilution Buffer. (E.g. 2 ml of 10X Standard / Sample Dilution Buffer + 19 ml of distilled water)
- 1X Wash buffer: Dilute 50X Wash buffer into distilled water to yield 1X Wash buffer. (E.g. 2 ml of 20X Wash buffer + 98 ml of distilled water) The diluted Wash buffer is stable for 4 weeks at 2°C to 8°C.
- Samples: Samples have to be diluted 1+100 with sample diluent. (e.g. Dispense 10µl sample and 1ml Sample Diluent Buffer into tubes to obtain

a 1+100 dilution and thoroughly mix with a Vortex.)

- **Standards:** Dilute 10 µl 50X Standards Buffer with 490 µl 1X Standard/Sample Dilution Buffer to yield a stock concentration of 2000 pg/ml. Keep the buffer in the vial for at least 15 min at RT to make sure the standard is completely dissolved before making serial dilutions. The standard diluent buffer serves as the zero standard (0 pg/ml), and the rest of the standard serial dilution can be diluted according to the suggested concentrations below: 2000 ng/ml, 1000 ng/ml, 500 ng/ml, 250 ng/ml, 125 ng/ml. DO NOT reuse the reconstituted **standard.**

Dilute Haptoglobin standard as according to the table below:

Standard	Haptoglobin Conc.	µl of Standard diluent	µl of standard
S5	2000 ng/ml	490 µl	10 µl (50X Standards Buffer)
S4	1000 ng/ml	300 µl	300 µl (S5)
S3	500 ng/ml	300 µl	300 µl (S4)
S2	250 ng/ml	300 µl	300 µl (S3)
S1	125 ng/ml	300 µl	300 µl (S2)
S0	0 ng/ml	300 µl	0 µl

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Standards and samples should be assayed in duplicates.

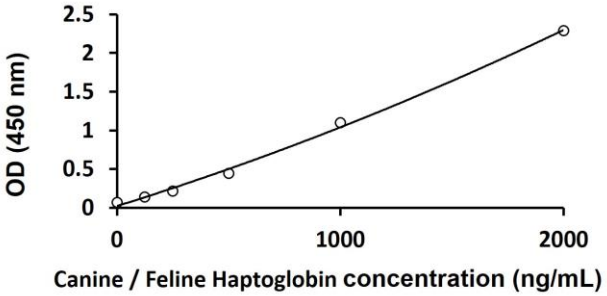
1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **100 µL** of Assay Buffer into the appropriate wells of the Antibody Coated Microplate.
3. Add **20 µL** of Standards, and prepared samples into each wells.
4. Incubate at RT for **60 minutes**.
5. Aspirate each well and wash, repeating the process 2 times for a total **3 washes**. Wash by filling each well with **1× Wash Buffer** (350 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
6. Add **100 µL** of HRP-Streptavidin buffer to each well, including the blank wells. Incubate at RT for **30 minutes** in the dark.
7. Aspirate each well and wash as step 5, but wash total for 5 times.
8. Add **100 µL** of TMB Substrate to each well, including the blank wells. Incubate at RT for **15-30 minutes** in the dark.
9. Add **100 µL** of **Stop Solution** to each well, including the blank wells. The color of the solution should change from blue to yellow.
10. Read the OD with a microplate reader at **450 nm** immediately. It is recommended reading the absorbance **within 10 minutes** after adding the stop solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
6. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as > 2000 ng/ml. For the calculation of the concentrations this dilution factor has to be taken into account.

EXAMPLE OF TYPICAL STANDARD VALUES

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The sensitivity of the Canine / Feline Haptoglobin ELISA Kit is 22.9 ng/ml.

Specificity

No interference with hemoglobin in hemolytic samples.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was ≤ 7.13% and CV value of inter-assay precision was ≤ 4.46%.

Recovery

Canine: 89%-107%

Feline: 88%-117%