TECOmedical Group

TECO®

Feline & Canine Haptoglobin ELISA

Feline & Canine Haptoglobin ELISA

Instructions for use
English

CE

Catalogue No. TE1033
For Research Use Only

always your partner

Symbol Description

Kit Instructions

Lot Number

Expiry Date

Storage Temperature

Manufacturer

Intended use

TE 1033

Caution: caustic

96
Tests

TECO*medical* AG
Headquarters **TECO***medical* Group

Gewerbestrasse 10
4450 Sissach
Switzerland

phone +41(0)61 985 81 00
fax +41(0)61 985 81 09

mail info@tecomedical.com
web www.tecomedical.com

Technical Services:

Germany phone	0800 985 99 99
France phone	0800 100 437
Benelux phone	+31(0)33 4951 473

Or contact our local representative in your country.

TECO® Feline & Canine Haptoglobin ELISA Kit

CONT **Reagents and Materials Supplied:**

Symbol	Description	Format
1	**96-well plate coated with Feline & Canine HPT Antibody** 12 break apart strips of 8 wells (12 x 8 in total), in a frame with cover plate. Ready to use.	**1 plate**
S	**Standard Stock 50 x** 100 µg/ml, tinted brown color	**1 x 0.2 ml**
2	**Wash Buffer 50 x**	**1 x 30 ml**
3	**Dilution Buffer 10 x**	**1 x 30 ml**
4	**Assay Buffer** Ready to use, tinted brown color	**1 x 12 ml**
6	**HRP Antibody Conjugate** Ready to use, tinted brown color	**1 x 12 ml**
7	**TMB Substrate** Ready to use.	**1 x 12 ml**
8	**Stop Solution – 1 M HCl** 1 M hydrochloric acid, ready to use.	**1 x 12 ml**
	**Kit instruction**	**1 x**

Storage
Store kit at 2–8 °C. Do not freeze. Store unused reagents at 2–8 °C.

Instructions for Use
The TECO® Feline & Canine Haptoglobin kit is a sensitive sandwich enzyme linked immunosorbent assay for the quantitative determination of haptoglobin in Feline and Canine serum.

Background
Haptoglobin, an acute phase protein, is part of immune system-mediated defence mechanisms found in the blood various animal species. Under normal conditions, haptoglobin is either absent from the blood or present at very low levels. However, haptoglobin can increase significantly in response to acute infection, inflammation or trauma.

Several functional properties of haptoglobin have been described. The major biological function of haptoglobin is to bind free hemoglobin in an equimolar ratio with very high affinity to prevent hemoglobin-mediated renal parenchymal injury and loss of iron following intravascular hemolysis[1]. The complex of haptoglobin with hemoglobin is metabolized in the hepatic reticuloendothelial system. Biosynthesis of haptoglobin occurs not only in the liver, but also in adipose tissue and lung, providing antioxidant and antimicrobial activity[2].

Haptoglobin can be used as a global parameter for inflammatory processes, since it is not disease-specific. This is underlined by several studies examining the haptoglobin serum levels in relation to different diseases or surgical interventions. Haptoglobin can be used as one of the major indexes in addition to other acute phase proteins like C-reactive protein and ceruloplasmin to examine the reaction in dogs[3, 4, 5]. In these studies, a slow increase in serum concentration of haptoglobin is described with maximum values two to seven days after surgery.

All studies strengthened the statement that haptoglobin is an alternative to serum amyloid A or fibrinogen as a diagnostic instrument for an acute phase reaction. In combination with serum amyloid A haptoglobin can be used to diagnose the inflammatory kinetic. Haptoglobin can as well be used to monitor the response of treatments.

[1] Lim SK (2001)
Consequences of haemolysis without haptoglobin.
Redox Rep 6: 375-378.

[2] Dobryszycka W (1997)
Biological Functions of Haptoglobin - New Pieces to an Old Puzzle.
Eur J Clin Chem Clin Biochem 35: 647-665.

[3] Conner JG, Eckersall PD, Ferguson J, Douglas TA (1988)
Acute phase response in the dog following surgical trauma.
Res Vet Sci 45: 107-110.

[4] Serin G, Ulutas PA (2010)
Measurement of serum acute phase proteins to monitor postoperative recovery in anoestrous bitches after ovariohysterectomy.
Vet Rec 166: 20-22.

[5] Tvarijonaviciute A, Martinez-Subiela S, Carrillo-Sanchez JD, Tecles F, Ceron JJ (2011)
Effects of orchidectomy in selective biochemical analytes in Beagle dogs.
Reprod Domest Anim 46: 957-963.

Assay Principle

The TECO® Feline & Canine Haptoglobin EIA Kit is a 96 well sandwich ELISA product. The assay uses affinity purified anti- haptoglobin antibodies immobilized at solid phase (microtiter wells). Pre-diluted samples are incubated in the microtiter wells for 60 min. The microtiter wells are subsequently washed, and horseradish peroxidase (HRP) conjugated antihaptoglobin antibodies are added and incubated for 30 minutes for detection. After incubation, the unbound HRP-labeled antibodies are washed away.

TMB substrate is added which reacts with the HRP and resulting in a concentration-depended color level. The reaction is stopped with HCl changing the blue color to yellow, and the plate is read using a plate reader at 450 nm.

Color development is proportional to the amount of haptoglobin in the sample.

Materials Required and not Supplied

- Pipette capable of accurately dispensing 10 -100 µl and 100 -1000 µl.
- Multichannel pipette for 100 µl
- Graduated cylinders for reconstituting or diluting reagents
- Automatic washer or equivalent plate washing system
- Distilled or deionized water
- Vortex mixer
- ELISA plate shaker (orbital shaker, 500 rpm)
- ELISA plate reader suitable for 96 well formats and capable of measuring at 450 nm (Reference Filter: 590–650 nm).
- ELISA plate reader software for data generation and analysis

Warnings and Precautions

This kit is intended for research use only.
Follow the instructions carefully.
Observe expiration dates stated on the labels and the specified stability for reconstituted reagents. Refer to "Materials Safety Data Sheet" for more detailed safety information.

TECOmedical AG is not liable for loss or harm caused by non-observance of the Kit instructions.

1. For Research Use Only. Not for use in diagnostic procedures.
2. Treat all specimen samples as potentially biohazardous material. Follow Universal Precautions when handling contents of this kit and any patient samples
3. Material of animal origin used in the preparation of this kit has been obtained from animals certified as healthy but these materials should be handled as potentially infectious.
4. Disposal of containers and unused contents should be performed in accordance with federal and local regulatory requirements.
5. Use the supplied reagents as an integral unit prior to the expiration date indicated on the package label.
6. Store assay reagents as indicated.
7. Do not use coated strips if pouch is punctured.
8. It is recommended to test each sample in duplicate.
9. Use of multichannel pipettes is recommended to ensure the timely delivery of liquids, however, do NOT use a multichannel pipette for plate washing steps.
10. a) 1 M hydrochloric acid is caustic and can cause severe burns.
    b) Handle TMB (3,3‘,5,5‘-tetramethylbenzidine) with care, and minimize exposure to light. Do not ingest. Avoid contact with skin, eyes, or clothing. If contact is made, wash with water. If ingested, call a physician.
11. As preservative 5-Bromo-5-nitro-1,3-dioxane (0,06 %) is used for the antibody and Sample Diluent. Do not ingest. Avoid contact with skin, eyes, or clothing. If contact is made, wash with water. If ingested, call a physician.

Preparation of Reagents

1 **Microwell Plate Coated with Feline & Canine HPT Antibody with plate cover**
12 break apart strips of 8 wells (96 in total) in a frame and sealed in a foil bag. Fit strip wells firmly into the frame. After opening, immediately return any unused wells to the original foil package and seal. Store at 2–8 °C until expiration date.

S **Standard Stock 50 x**
1 vial containing 0.2 ml HPT (100 µg/ml)
Store at 2–8 °C until expiration date.
See Standard preparation.

2 **Wash Buffer 50 x**
1 vial of 30 ml buffer, 50 x concentrated. Precipitation may occur in the buffer; resolve before use by warming up and mixing. Bring the vial content to 1500 ml with deionized or distilled water. The diluted washing solution is stable for 4 weeks at 2–8 °C. Store undiluted buffer at 2–8 °C until expiration date.

3 **Dilution Buffer 10 x**
1 vial of 30 ml. 10 x concentrated. Dilute 1:10 with deionized or distilled water e.g. 30 ml + 270 ml deionized or distilled water. The diluted solution is stable for 4 weeks at 2–8 °C. Store undiluted buffer at 2–8 °C until expiration date.

4 **Assay Buffer**
1 vial of 12 ml. Ready to use. Store at 2–8 °C until expiration date.

6 **HRP Antibody Conjugate**
1 vial of 12 ml. Ready to use. Store at 2–8 °C until expiration date.

7 **TMB Substrate**
1 vial of 12 ml of H_2O_2 and stabilized 3,3‘,5,5‘-tetramethylbenzidine. Ready to use.
Store at 2–8 °C until expiration date.

8 **Stop Solution – 1 M HCl**
1 vial of 12 ml of 1 M hydrochloric acid. Ready to use.
Store at 2–8 °C until expiration date.

Preparation and Stability of Serum Samples

Sample Type and Preparation: Serum, Plasma and Cell Culture
Non-lipemic samples are recommended. Centrifuge collected blood samples within 4 hours. Predilute samples 1:10 000 with Dilution Buffer 1 x 3 in two dilution steps.

Step	Dilution Factor	Dilution Buffer
1	100	990 µl Dilution Buffer + 10 µL Sample
2	10000	990 µl Dilution Buffer + 10 µL Sample (1:100)

Sample Stability
- Maximum 3 days at 2–8 °C or room temperature
- Maximum 12 months at -20 °C
- Longer Storage at -80 °C
- Maximum 3 freeze/thaw cycles

Preparation of Standard (in Dilution Buffer)
Standards have to be prepared freshly before use.
Use the Dilution Buffer 1 x 3 delivered by TECOmedical for Standard preparation.

The Standard vial S contains 100 µg/ml Feline & Canine HPT.
Preparation of the standard curve **with Dilution Buffer.**

ID	Concentration	Dilution Buffer
Std A	2000 ng/mL	980 µl Dilution Buffer + 20 µL Standard Stock
Std B	1000 ng/mL	300 µL Dilution Buffer + 300 µL Std A
Std C	500 ng/mL	300 µL Dilution Buffer + 300 µL Std B
Std D	250 ng/mL	300 µL Dilution Buffer + 300 µL Std C
Std E	125 ng/mL	300 µL Dilution Buffer + 300 µL Std D
Std F	0 ng/mL	Dilution Buffer

Assay Procedure

It is recommended that all determinations (standards and samples) are assayed in duplicate. When performing the assay, standards and samples should be pipetted as fast as possible (< 15 minutes).
To avoid distortions due to differences in incubation times, Substrate Solution and Stop Solution should be added to the plate in the same order and with the same time interval. Before use, allow all reagents to stand at room temperature (20–25 °C) for at least 30 minutes. During all incubation steps, plates should be sealed with the adhesive foil or a plastic cover. For light protection, incubate in a dark chamber or cover plate with aluminium foil.

1. Allocate the wells of the Microwell Plate for standards and samples.

2. Pre-wash the microassay strips as follows:
   a. Using a wash bottle or automated plate washing device,
      add approximately 350 µL Wash Buffer to each well.
   b. Incubate the wells for two minutes at 20-25°C.
   c. Aspirate the contents from each well.
      Invert the plate and tap firmly on absorbent paper to remove any remaining liquid.

3. Add **100 µl** Assay Buffer to each well (multichannel pipette)

4. Pipette **20 µl** of each standard (A till F) and diluted samples into the corresponding wells.

5. Incubate the plate for **1 h** at room temperature (20–25 °C) on a shaker (500 rpm).

6. After incubation, aspirate the wells by using a plate washer or manually decant by inverting the plate. Wash the wells 3 times with 350 µl diluted Wash Buffer per well. After the last wash cycle tap the inverted wells on a dry absorbent surface to remove excess wash solution. The use of an automatic plate washer is recommended.

7. Add **100 µl** of HRP Antibody Conjugate (multichannel pipette).

8. Incubate the plate for **30 min** in the dark at room temperature (20–25 °C) on a shaker (500 rpm).

9. After incubation, aspirate the wells by using a plate washer or manually decant by inverting the plate. Wash the wells 5 times with 350 µl diluted Wash Buffer per well. After the last wash cycle tap the inverted wells on a dry absorbent surface to remove excess wash solution. The use of an automatic plate washer is recommended.

10. Pipette 100 µl of the TMB Substrate Solution in each well (multichannel pipette).

11. Incubate the plate for **15–30 min** in the dark at room temperature (20–25 °C) on a shaker (500 rpm).

12. Stop the reaction by adding **100 µl** of Stop Solution (multichannel pipette).

13. Measure the color reaction within **10 min** at 450 nm (reference filter between 590–650 nm).

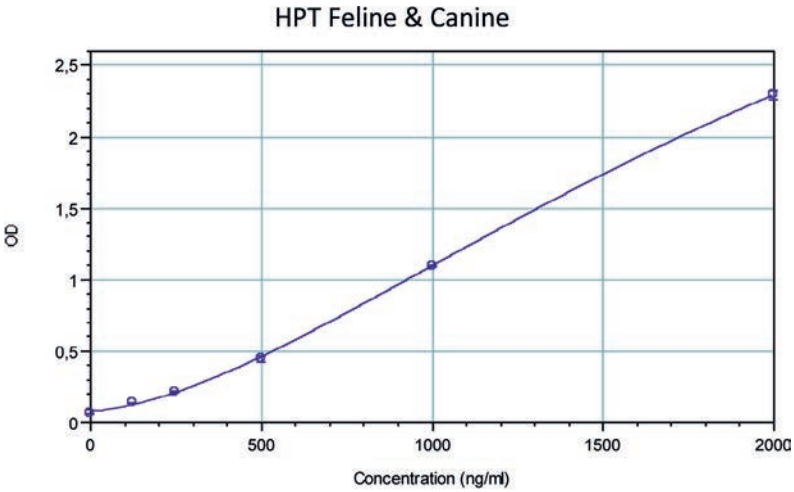
Result Analysis

Feline and Canine Haptoglobin Concentration
A Standard curve can be established by plotting standard concentration on the x-axis (linear scale) against the absorbance of the Standards on the y-axis (linear scale). A 4-parameter curve fit should be used for automatic data reduction. The feline or canine HPT concentration of samples will be obtained by multiplying the value read off the standard curve by the dilution factor used for the given sample.

Samples with higher absorbance values than Standard A should be tested again with a higher dilution.

Typical Standard Curve
(Example only, not for use in calculation of actual results)

Standards	Concentration (ng/ml)	Absorption at 450 nm
Standard A	2000 ng/mL	2.288
Std B	1000 ng/mL	1.097
Std C	500 ng/mL	0.445
Std D	250 ng/mL	0.215
Std E	125 ng/mL	0.135
Std F	0 ng/mL	0.067



Interferences
No interference with hemoglobin in hemolytic samples.

Detection Limit
The mean detection limit is defined as Standard F (0 ng/ml) plus 3 SD: 22.9 ng/ml.

Test Performance

Precision
(Inter assay)

Sample (serum)	Mean (ng/ml)	SD (ng/ml)	CV (%)
Sample 1 Canine	186	18.9	10.2
Sample 2 Canine	350	20.0	5.7
Sample 3 Canine	739	44.1	6.0
Sample 4 Feline	683	45.1	6.6

(Intra assay)

Sample (serum)	Mean (ng/ml)	SD (ng/ml)	CV (%)
Sample 1 Canine	186	11.7	6.2
Sample 2 Canine	350	17.9	5.1
Sample 3 Canine	739	27.7	3.7
Sample 4 Feline	683	19.7	2.9

Spike Recovery

The recovery of HPT spiked to normal feline & canine samples was 106 %.

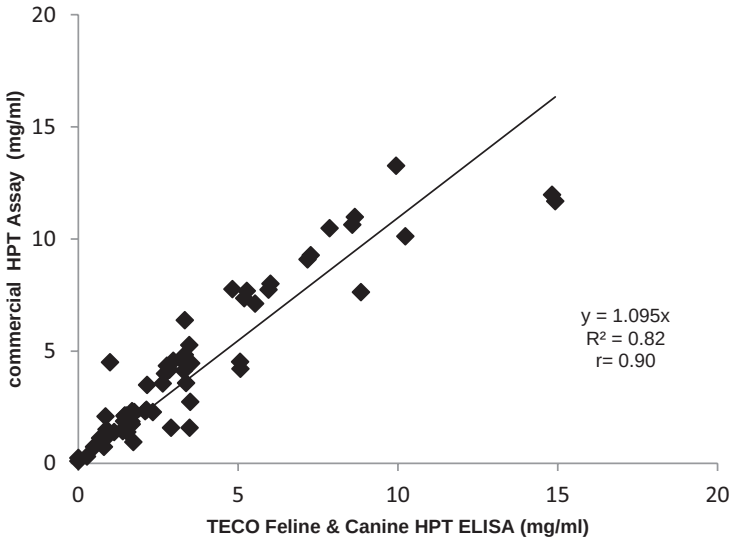
Sample	Concentration ng/ml	Added ng/ml	Measured ng/ml	Expected ng/ml	Spike Recovery %
Sample 1 Canine	196	370	570	567	101
Sample 2 Canine	359	370	743	729	102
Sample 3 Canine	807	370	1286	1177	109
Sample 4 Feline	879	370	1395	1249	112
Sample 5 Canine	399	370	825	769	107
Sample 6 Feline	842	370	1240	1212	102

Dilution Recovery

The mean dilution recovery of feline & canine samples was 106 %.

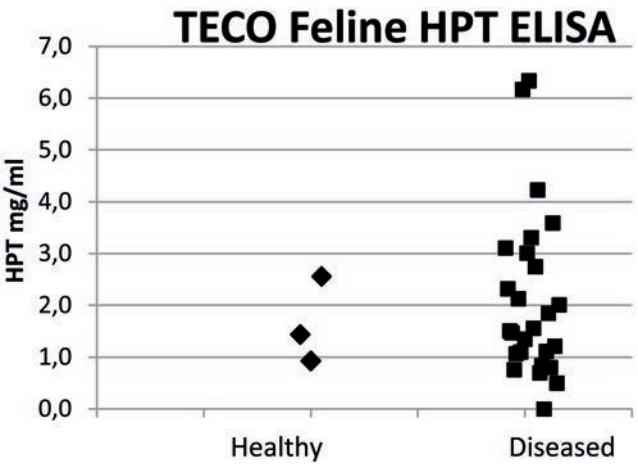
Sample	Dilution	Measured ng/ml	Expected ng/ml	Recovery %
Sample 1 Canine	5000 10000 20000	368 196 118	 184 92	 107 128
Sample 2 Canine	5000 10000 20000	772 359 187	 386 193	 93 97
Sample 3 Canine	5000 10000 20000	1758 807 390	 879 440	 92 89
Sample 4 Feline	5000 10000 20000	1862 879 407	 931 466	 94 87
Sample 5 Canine	5000 10000 20000	905 399 193	 452 226	 88 85
Sample 6 Feline	5000 10000 20000	1291 842 379	 645 323	 130 117

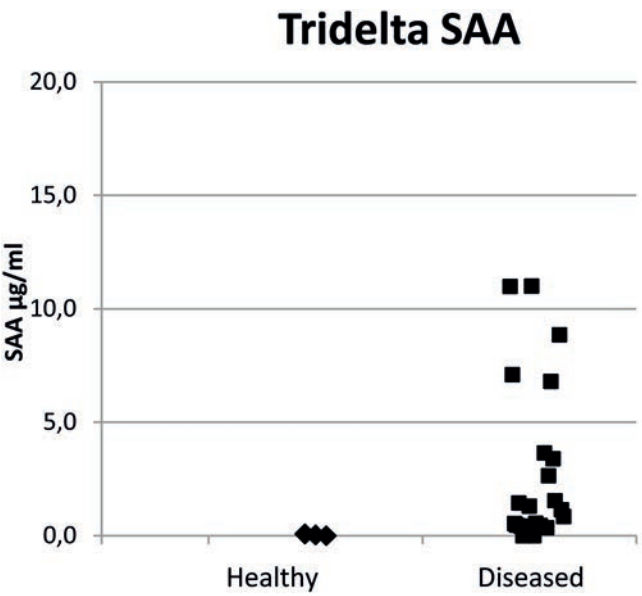
Correlation between TECOmedical Feline & Canine Assay and a commercial HPT Assay



Feline HPT ELISA Results

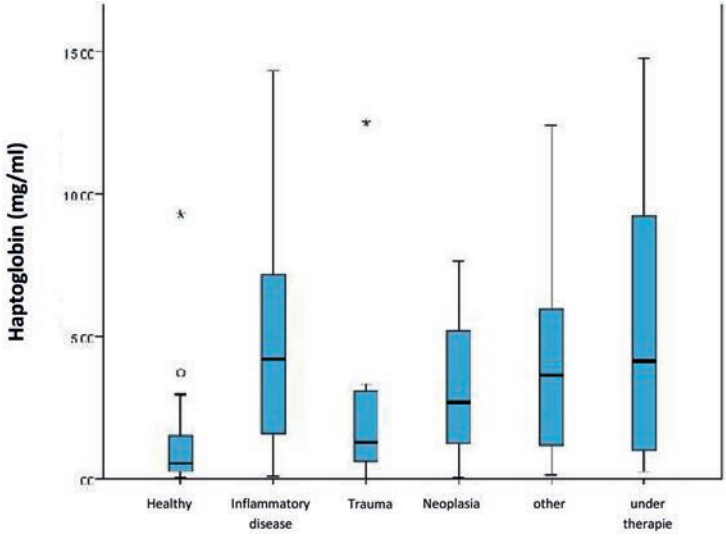
Observed Values
Serum samples of 9 healthy cats were tested.
The mean haptoglobin concentrations were 1.7 mg/ml +/- 0.6.
Based on these values a cut off of 3.0 mg/ml (mean + 2SD) has been defined.





Canine HPT ELISA Results

Comparison of haptoglobin median, dependent on different diseases



Observed Values

Serum samples of 20 healthy dogs were tested.
The mean haptoglobin concentrations were 1.1 mg/ml +/- 0.8

Based on these values a cut off of 2.7 mg/ml (mean + 2SD) has been defined.

Note
All data Normal Range and disease states
B. Kohn et al., 2012, Clinic of Small Animals, Faculty of Veterinary Medicine
Freie Universität Berlin – Germany

TECO® Feline & Canine Haptoglobin ELISA

Assay Procedure – Quick Guide

- Bring samples and reagents to room temperature. Mix the samples well.
- Dilution Buffer: Dilute1:10 with deionized or destilled water.
- Dilute Feline & Canine HPT Standard S according to instruction with Dilution Buffer 1x.
- Washing Buffer: Dilute 1:50 with deionized or destilled water.
- Predilute samples with Dilution Buffer 1 x (e.g. 1:10 000).

Prepare the required number of Assay Strips.

Immediately before use pre-wash the microassay strips once with **350 µl** with **2 min**. soake time, aspirate and tap the inverted wells on a clean dry absorbent surface.

Pipette **100 µl** diluted Assay Buffer into each well.
Add **20 µl** standards and diluted samples into assay wells.

Incubate **1 h** at 20–25 °C on a shaker (500 rpm).

Aspirate and wash **3 x** with **350 µl** Wash Buffer, aspirate and tap the inverted wells on a clean dry absorbent surface.

Pipette **100 µl** HRP Antibody Conjugate into each well.

Incubate **30 min** at 20–25 °C in the dark on a shaker (500 rpm).

Aspirate and wash **5 x** with **350 µl** Wash Buffer, aspirate and tap the inverted wells on a clean dry absorbent surface.

Pipette **100 µl** TMB Substrate Solution.

Incubate **15–30 min** at 20–25 °C in the dark on a shaker (500 rpm).

Pipette **100 µl** Stop Solution.

Read the Optical Density at **450 nm**, using a referencefilter between 590-650 nm. Analyze the assay results using a 4- parameter curve fit: $y= (A-D)/(1+(x/C)^B)+D$
Calculations: measured value x dilution

Please read Kit instruction before using the Quick Guide.