



Canine and Feline Haptoglobin (cfHAPT) BIO-ANALYSER

Product Number: RL0008

Assay Precautions

- Do not use kit or individual reagents past their expiry date.
- Do not mix or substitute reagents from different kit batches.
- Samples should be stored refrigerated or frozen if they are not to be analysed shortly after collection.
- Avoid repeat freeze/thawing of samples.
- Where possible avoid the use of haemolysed or lipaemic serum.
- Cover or cap all reagents when not in use unless stored on board analyser.
- Use clean and preferably disposable labware for all reagent preparation.
- Care must be taken not to contaminate reagents. Use fresh tips for each sample and reagent.

Safety Precautions

- For *in vitro* research purposes only.
- Dispose of all clinical samples, infected or potentially infectious material in accordance with good laboratory practice.
- Wear disposable gloves and safety glasses where appropriate.
- The kit contains reagents that may cause irritation to skin and eye. Any reagent which comes into contact with the skin or eye should be washed off with water immediately.
- The kit contains reagents that may cause irritation to respiratory or gastrointestinal tract if inhaled or ingested. Seek medical attention if you feel unwell.



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Intended Use

The canine and feline Haptoglobin (cfHAPT) turbidimetric assay is intended for the quantitative measurement of haptoglobin in canine serum.

Clinical Use

Acute phase proteins (APPs) such as haptoglobin are serum proteins that increase in concentration 24 to 48 hours following infection, inflammation, or trauma. The circulating concentrations of these proteins can provide an objective measure of the health status of an animal and are increasingly being used as markers of animal health and welfare.

Serum concentrations of APPs are related to the severity of the underlying condition, and provide a ready means of evaluating both the presence and extent of disease.

In a healthy animal, HAPT is present in very low levels ranging from less than or equal to 3.0 mg/mL in canines and less than or equal to 3.5 mg/mL in felines.

Methodology

The cfHAPT bio-analyser assay is an immunoturbidimetric assay. HAPT in a sample of canine or feline serum is captured by Reagent 1 and 2 to form an antibody-antigen complex.

The mixture is then measured turbidimetrically by the spectrophotometer in the biochemical analyser. The absorbance is directly proportional to the amount of cfHAPT present in the serum sample.

The assay is calibrated by comparison to standards with known concentrations of Haptoglobin. It is recommended that a calibration is performed once every 8 weeks, with quality controls (QCs) run once a day.

Reagents Provided

- Reagent 1 (R1)
 - 1 x 40 mL
- Reagent 2 (R2)
 - 1 x 10 mL
- Calibrator
 - 3 x 0.4 mL
- QC set
 - High and Low 20 x 100 µL

Additional Materials Required

- Biochemical analyser, preferably an Olympus AU series.
- Sample cups and racks.
- A variety of micropipettes and disposable tips capable of dispensing 10 µL – 1000 µL.
- Distilled water.

Sample Preparation

The serum should be separated from the red blood cells as soon as possible after collection. Samples should be frozen if analysis cannot be performed immediately.



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Storage and Stability

The contents of the canine and feline HAPT bio-analyser kit should be stored at 2-8°C and used within the expiry date detailed on packaging.

Individual components are labelled with individual expiry dates; however the kit should be used within expiry date detailed on packaging.

Reagent Preparation

- R1 and R2 reagents are provided ready to use.

For use, open (a record can be made of the open date) and place bottles directly onto the biochemical analyser.

- The calibrator is provided as a stock and requires diluting as directing on the calibrator label. Prepare remaining levels by serial dilution as below:

The HAPT standard should be diluted in purified water as detailed below

- Label 6 tubes S1-S6. Add 150 µL of purified water to tubes S2-S6.
- Invert HAPT standard several times before use and add 150 µL to tube S1.
- Add 150 µL HAPT standard to tube S2. Mix well and serially dilute as directed in table 1.

Table 1: Preparation of Standard Curve

Standard tube number	HAPT concentration (mg/mL)	Volume of HAPT Standard S1 (µL)	Volume of purified Water (µL)	Serial Dilution
S1	3.28	150	-	-
S2	1.64	150	150	-
S3	0.82	-	150	150µL of S2
S4	0.41	-	150	150µL of S3
S5	0.21	-	150	150µL of S4
S6	0.00	-	150	

For use, remove from storage, dispense required volume into sample cup and return to storage.

- QC set contains a Low and High QC.

For use, remove from storage, carry out dilution as stated on label of QC bottle and return to storage.

Procedure

- Place R1 reagent and R2 reagent onto the biochemical analyser.
- Dispense the required volume of calibrator and QCs.
- Dispense distilled water into the cup of the blank bio-analyser rack.
- Perform calibration and QCs;
 - If QCs are within range dispense sample into sample cup, add to analyser and analyse.
 - If QCs are outside range re-run the QCs.
 - If QCs remain outside range re-calibrate and re-run QCs.
- Dilute serum samples 1 in 2 in distilled water.
- In the event of samples measuring below the curve samples can be re-run neat. Linearity of dilution has been confirmed to 1 in 32.

QC Acceptance Ranges

Please see label associated with each QC for batch specific ranges.

NB – The stated ranges are applicable for Olympus AU series biochemical analysers. For any other analyser data, please contact us.



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Performance Characteristics

1. Assay Range

0.21 – 3.28 mg/mL

2. Intra-Assay Reproducibility

Two canine pools were assayed in replicates of five in a single run to determine intra (within) assay reproducibility.

	Pool 1	Pool 2
n	5	5
Mean (ng/mL)	0.3	3.02
Standard Deviation	0.004	0.158
%CV	1.3	5.2

Mean intra-assay variation: 3.3%.

3. Inter-Assay Reproducibility

Two canine pools were assayed in replicates of five on four separate occasions to determine inter-assay reproducibility.

	Pool 1	Pool 2
n	20	20
Mean (ng/mL)	0.3	2.94
Standard Deviation	0.013	0.102
%CV	4.5	3.5

Mean inter-assay variation: 4.0%.

4. Sensitivity

0.25 mg/mL.

5. Dilution linearity

1 in 1 - 1 in 32.

Sample should initially be measured at a 1 in 2 dilution and can then be repeated neat if below the assay sensitivity.

Analyser Parameters

Olympus AU Series

General

	Volume (µl)		Pre-dilution Rate
Sample	5	-	10
R1	83	-	-
R2	30	-	-

Primary Wavelength	Secondary Wavelength
340nm	0nm

Method	Reaction Slope
End	+

Measuring point 1		Measuring point 2	
First	Last	First	Last
11	27	-	-

Reagent OD Limit			
First L	Last L	First H	Last H
-2	-2	2.5	2.5

Dynamic Range	
L	H
0.25	4

Correlation Factor		On board stability period
A	B	
1	0	Under assessment

CALIBRATION		
Cal Type	Measure Type	Formula
5AB	Rack	POLYGONAL