TECOmedical Group

TECO®
Canine CRP ELISA

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Instructions for use
English

Catalogue No. TE1024
For Research Use Only

always your partner

Symbol Description

Kit Instructions

Lot Number

Expiry Date

In vitro Diagnostic

Storage Temperature

Manufacturer

Tests

TE 1024

Attention

Intended use

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TECO® Canine CRP ELISA Kit

CONT Reagents and Materials Supplied:

Symbol	Description	Format
1	**96-well plate coated with canine CRP specific antibody with plate cover** 12 break apart strips of 8 wells (12 x 8 in total), in a frame, Ready to use	**1 plate**
A	**Standard Stock Solution** 500 ng in 100 µl	**1 x 0.4 ml**
L	**Control 1** Range as indicated on data sheet	**1 x 0.05 ml**
H	**Control 2** Range as indicated on data sheet	**1 x 0.05 ml**
2	**Wash Buffer** 50 times concentrated	**1 x 30 ml**
3	**Sample Diluent** Ready for use	**1 x 100 ml**
4	**Assay Buffer** Ready for use	**1 x 12 ml**
6	**HRP Antibody Conjugate** Ready for use	**1 x 12 ml**
7	**TMB Substrate** Ready for use	**1 x 12 ml**
8	**Stop Solution – 1 M HCl** 1 M hydrochloric acid, ready for 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® Canine CRP kit is a sensitive sandwich enzyme linked immunosorbent assay for the quantitative determination of CRP in canine serum, plasma and cell culture.

Background
The measurement of CRP is used for diagnosis and follow-up of inflammatory diseases. Although CRP is not disease-specific, the detection of an increased CRP level provides a valuable indication of the presence of inflammatory processes. CRP levels in serum/blood rise within hours after the onset of inflammation and reach a peak during the acute phase. As soon as the inflammation subsides, the CRP levels begin to decrease. As the erythrocyte sedimentation rate may be influenced by other physiological processes, the determination of CRP is a more reliable and more sensitive indicator of inflammation for:

- Rapid and easy detection of inflammatory processes
- Monitoring of therapy (Immune-mediated inflammatory processes)
- Screening before and follow-up after surgery
- Helpful as an aid in the diagnosis of infectious diseases
- Useful as part of health checks – especially older dogs
- Screening of health status e. g. before travelling or vaccination
- Pregnancy
- Cancer

Assay Principle

The TECO® Canine CRP EIA Kit is a 96 well immuno-capture ELISA product. Samples are incubated with a specific polyclonal antibody coated on the plate. After incubation, the unbound material is washed away. Then a HRP linked polyclonal antibody that specifically recognizes canine CRP is added to the wells. After a further incubation and washing step, a TMB substrate is added which reacts with the HRP and resulting in a concentration-depended color level. The reaction is stopped with HCl and the plate is read using a plate reader at 450 nm.

Color development is proportional to the amount of canine CRP in the sample.

Materials Required and not Supplied

- Pipettes capable of accurately dispensing 20 µl, and 100 to 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 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 isn´t 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 Canine CRP Specific Antibody**
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.

A **Standard**
1 vial containing 0.4 ml canine CRP (500 ng/100 µl).
Store at 2–8 °C until expiration date.

L **Control 1**
1 vial of 0.05 ml low control. Range see data sheet.
Store at 2–8 °C until expiration date.

H **Control 2**
1 vial of 0.05 ml high control. Range see data sheet.
Store at 2–8 °C until expiration date.

2 **Wash Buffer**
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 **Sample Diluent**
1 vial of 100 ml. Ready for use. Store at 2–8 °C until expiration date.

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

6 **HRP Antibody Conjugate**
1 vial of 12 ml. Ready for 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 for 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 for 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 1:100 with Sample Diluent as well as controls (10 µl Sample + 990 µl Sample Diluent).

Note

To measure very low concentration (< 5 mg/l) sample and control dilution of 1:50 or 1:25 with Sample Diluent. Lower dilution than 1:25 are not recommended.
To measure concentrations expected higher than 50 mg/l, sample should be used at a higher dilution (e.g. 1:200 or 1:500).

Stability

Serum samples are stable for 72 hours at room temperature, for 5 days at 2–8 °C, longer storage at -20 °C or at -70 °C. Maximum 3 freeze- and thaw cycles.

Preparation of Standard (in Sample Diluent)

Standards have to be prepared freshly before use.
Use the Sample Diluent 3 delivered by TECOmedical for Standard preparation.

The Standard vial A contains 500 ng canine CRP in 100 µl.
Preparation of the standard curve **with Sample Diluent.**

ID	Concentration	Sample Diluent
Std A	500 ng/mL	900 µl Sample Diluent + 100 µL Standard Stock Solution
Std B	278 ng/mL	400 µL Sample Diluent + 500 µL Std A
Std C	154 ng/mL	400 µL Sample Diluent + 500 µL Std B
Std D	86 ng/mL	400 µL Sample Diluent + 500 µL Std C
Std E	48 ng/mL	400 µL Sample Diluent + 500 µL Std D
Std F	0 ng/mL	Sample Diluent

Assay Procedure

It is recommended that all determinations (Standards, Controls and samples) are assayed in duplicate. When performing the assay, Standards, Controls 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 1 for Standards, Controls and samples.

2. Add **100 µl** Assay Buffer 4 to each well (multichannel pipette).

3. Pipette **20 µl** of each Standard (A till F), diluted Controls (L and H) and diluted samples into the corresponding wells.
   Note: Controls have to be diluted like samples, e. g. 1 : 100 (see Note page 8)

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

5. After incubation, aspirate the content of the wells and wash 3 times with 350 µl diluted Wash Buffer 2 The use of an automatic plate washer is recommended.

6. Add **100 µl** of HRP Antibody Conjugate 6 (multichannel pipette).

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

8. After incubation, aspirate the content of the wells and wash 5 times with 350 µl diluted Wash Buffer 2 The use of an automatic plate washer is recommended.

9. Pipette 100 µl of the TMB Substrate Solution 7 in each well (multichannel pipette).

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

11. Stop the reaction by adding **100 µl** of Stop Solution 8 (multichannel pipette).

12. Measure the colour reaction within 10 minutes at 450 nm (reference filter between 590–650 nm).

Protocols for the different automatic ELISA systems are available.

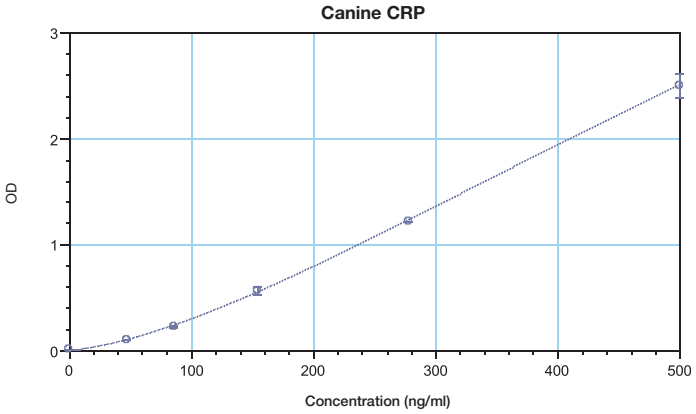
Result Analysis

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. Alternatively, a quadratic (polynomial) fit is possible. The canine CRP concentration of samples will be obtained by multiplying the value read off the standard curve by the dilution factor used for the given sample.
For each assay, the results of the Controls must be within the target range indicated for every lot.
The QC protocol with target ranges is provided with the kit. If control values are not within the limits of the provided range, the assay results should be considered questionable and the samples should be tested again.

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

Typical Standard Curve and Controls
(Example only. Not for use in calculation of actual results)

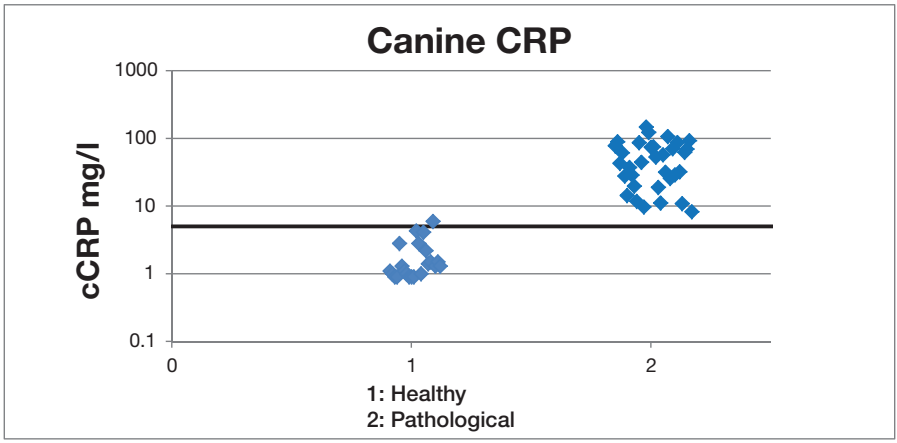
Standards	Concentration (ng/ml)	Absorption at 450 nm
Stock Standard A	500	2.632
Std B	278	1.413
Std C	154	0.645
Std D	86	0.254
Std E	48	0.121
Std F	0	0.014



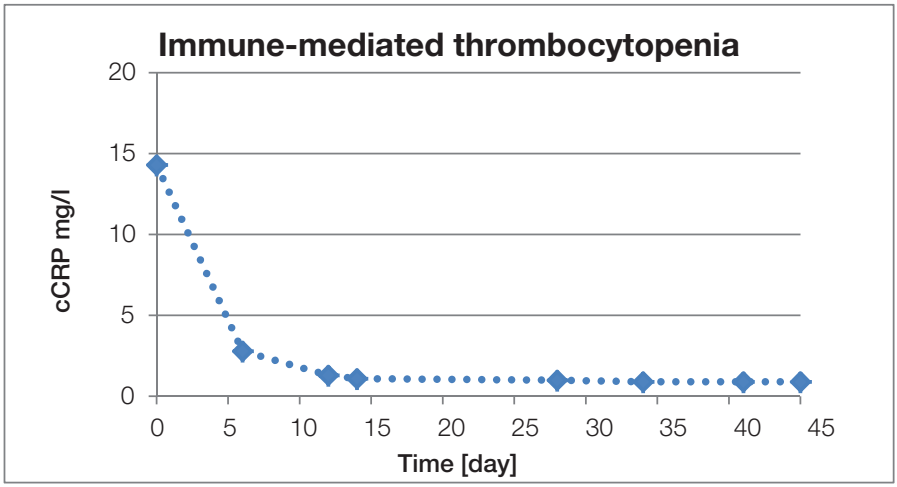
Curve Fit Option – Fixed Weight Value

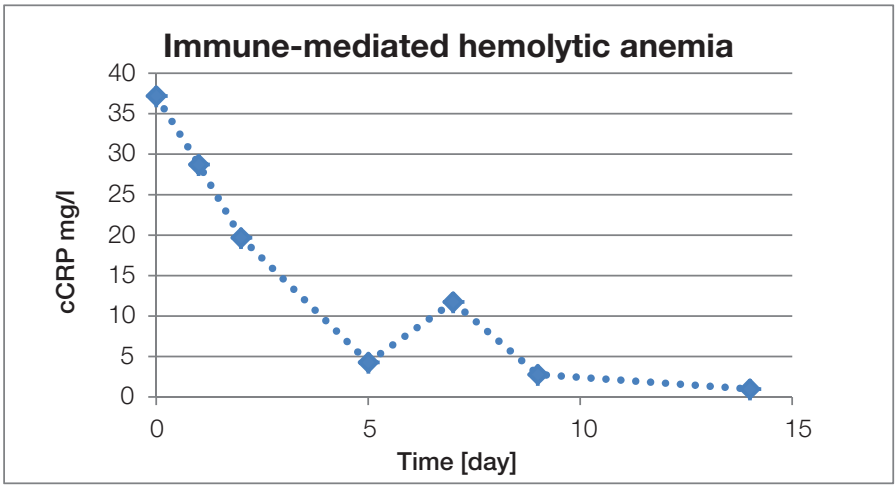
Observed Values

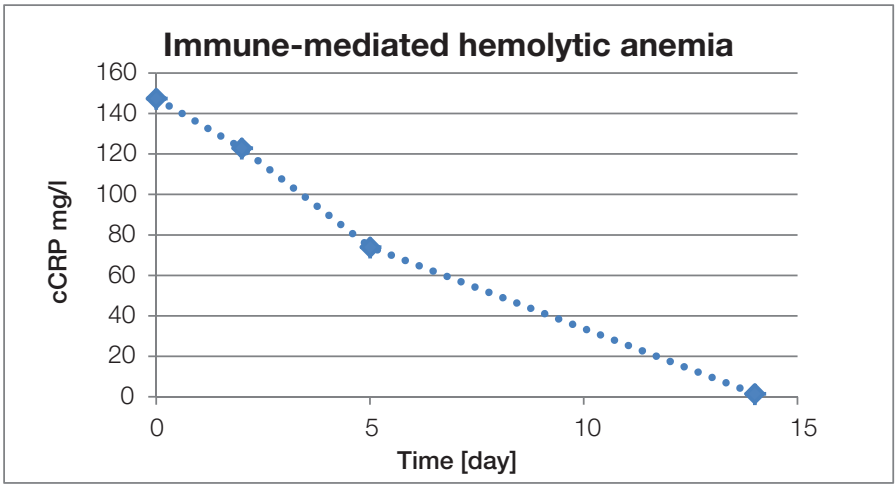
Cut-off: CRP in healthy canine < 10 mg/l.



Therapy Monitoring







Note
Data Normal Range and Therapy monitoring:
B. Kohn et al., 2011, Clinic of Small Animals, Faculty of Veterinary Medicine
Freie Universität Berlin – Germany

Test Performance

Precision

Inter assay

Sample (serum)	Mean (mg/l)	SD (mg/l)	CV (%)
Sample 1	36.6	1.2	3.4
Sample 2	28.9	1.2	4.1
Sample 3	24.8	1.0	4.2
Sample 4	42.4	1.6	3.7

Intra assay

Sample (serum)	Mean (mg/l)	SD (mg/l)	CV (%)
Sample 1	36.7	1.7	4.7
Sample 2	29.1	1.7	5.7
Sample 3	24.8	1.1	4.3
Sample 4	42.5	1.8	4.2

Detection Limit

The kit detection limit was calculated in 15 Runs.
The mean detection limit is defined as STD F (0 ng/ml) plus 3 SD: 29.8 ng/ml.

Spike Recovery

The recovery of Dog CRP spiked to normal samples was 103 %.

Sample	Concentration (mg/l)	Added (mg/l)	Expected (mg/l)	Measured (mg/l)	Spike Recovery (%)
1	0	28.2 15.3 7.4	28.2 15.3 7.4	28.0 15.1 7.5	**99** **99** **101**
2	0	28.2 15.3 7.4	28.2 15.3 7.4	29.2 15.2 8.4	**104** **99** **114**
3	0	28.2 15.3 7.4	28.2 15.3 7.4	28.5 15.1 7.5	**101** **99** **101**

Dilution Recovery

Sample	Measured (mg/l)	Dilution	Expected (mg/l)	Dilution Recovery (%)
1	33,6	1		
	16,9	2	16,8	**101**
	8,7	4	8,4	**104**
	4,4	8	4,2	**105**
2	32,0	1		
	15,7	2	16,0	**98**
	8,3	4	8,0	**104**
	3,7	8	4,0	**93**
3	34,4	1		
	17,3	2	17,2	**101**
	9,5	4	8,6	**110**
	4,8	8	4,3	**112**
4	39,4	1		
	20,5	2	19,7	**104**
	10,6	4	9,9	**108**
	5,4	8	4,9	**110**
5	30,0	1		
	14,5	2	15,0	**97**
	6,8	4	7,5	**91**

Species specifity

No cross reaction occurred with different species serum

mice	goat	horse
rat	bovine	chicken
sheep	pig	human

Interferences

Hemoglobin/hemolysate — no interference

Notes:

TECO® Canine CRP ELISA

Assay Procedure – Quick Guide

- Bring samples and reagents to room temperature. Mix the samples well.
- Dilute 100 µl Canine CRP A with 900 µl Sample Diluent.
- Washing Buffer: Dilute 1:50 with Aqua dest.
- Predilute Controls and samples with Sample Diluent (e.g. 1:100).

Prepare the required number of Assay Strips

Pipette **100 µl** Assay Buffer into each well

Pipette **20 µl** Standards A – F, diluted Controls L + H and diluted samples

Incubate **60 min** at 20–25 °C on a shaker (500 rpm)

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

Pipette **100 µl** HRP Antibody Conjugate 6 into each well

Incubate **30 Minutes** at 20–25 °C on a shaker (500 rpm)

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

Add **100 µl** TMB Substrate 7 into each well

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

Add **100 µl Stop Solution** 8 into each well

Measure the absorbance at 450 nm
Quantification software, 4-parameter fit: y = (A-D)/ (1+(x/C) ^B) +D)
Reference measurement should be performed at 590–650 nm.

Please read Kit instruction before using the Quick Guide