

VetLine Canine C-Reactive Protein (cCRP)

ELISA

Only for veterinary in-vitro diagnostic use.

Enzyme immunoassay for the quantitative determination of Canine C-Reactive Protein in canine serum.

Instructions for use

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Product Number: CCRPVT4050 (96 Determinations)

ENGLISH

1. INTENDED USE

The VetLine Canine C-Reactive Protein ELISA is intended for the quantitative determination of Canine C-Reactive Protein in canine serum.

2. PRINCIPLE OF THE ASSAY

The quantitative immunoenzymatic determination of specific cCRP is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.

Microtiterplates are coated with specific antibodies to bind corresponding cCRP of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured cCRP. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.

The intensity of this product is proportional to the amount of cCRP in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA microwell plate reader.

3. MATERIALS

3.1. Reagents supplied

- **Microtiterplate:** 12 break-apart 8-well snap-off strips coated with anti-Canine C-Reactive Protein antibodies; in resealable aluminium foil.
- **Sample Dilution Buffer:** 1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; white cap; ≤ 0.0015 % (v/v) CMIT/MIT (3:1).
- **Stop Solution:** 1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use; red cap.
- **Washing Buffer (20x conc.):** 1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap; 0.2% (w/v) 5-Bromo-5-nitro-1,3-dioxane.
- **Conjugate:** 1 bottle containing 15 mL of peroxidase labelled anti-Canine C-Reactive Protein antibodies; coloured red; ready to use; white cap.
- **TMB Substrate Solution:** 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1 %; ready to use; yellow cap.
- **Standards:** 6 vials, each containing 2 mL standard (Canine C-Reactive Protein); coloured yellow; ready to use; ≤ 0.02 % (v/v) MIT.

Standard A:	0	ng/mL; yellow cap
Standard B:	10	ng/mL; yellow cap
Standard C:	16	ng/mL; yellow cap
Standard D:	30	ng/mL; yellow cap
Standard E:	50	ng/mL; yellow cap
Standard F:	75	ng/mL; yellow cap

For hazard and precautionary statements see 11.1.

3.2. Materials supplied

- 1 Cover foil
- 1 Instructions for use (IFU)

3.3. Materials and Equipment needed

- ELISA microwell plate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37 °C
- Manual or automatic equipment for rinsing wells
- Pipettes to deliver volumes between 10 and 1000 µL
- Vortex tube mixer
- Distilled water
- Disposable tubes

4. STABILITY AND STORAGE

Store the kit at 2...8 °C. The opened reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

5. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20...25 °C) and mix them before starting the test run!

5.1. Microtiterplate

The break-apart snap-off strips are coated with anti-Canine C-Reactive Protein antibodies. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

5.2. Washing Buffer (20x conc.)

Dilute Washing Buffer 1 + 19; e. g. 10 mL Washing Buffer + 190 mL distilled water. The diluted buffer is stable for 5 days at room temperature (20...25 °C). In case crystals appear in the concentrate, warm up the solution to 37 °C e.g. in a water bath. Mix well before dilution.

5.3. TMB Substrate Solution

The reagent is ready to use and has to be stored at 2...8 °C, away from the light. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

6. SAMPLE COLLECTION AND PREPARATION

Use canine serum samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70...-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.

Heat inactivation of samples is not recommended.

6.1. Sample Dilution

Before assaying, all samples should be diluted 1+1000 with Sample Dilution Buffer. To avoid pipetting errors, a pre-dilution should be prepared. For example, mix 10 µl of sample with 100 µl of Sample Dilution Buffer to make a pre-dilution. In a further step, 10 µl of this pre-dilution is mixed with 1 mL of Sample Dilution Buffer to obtain a 1+1000 dilution. All steps should be thoroughly mixed with a vortex.

7. ASSAY PROCEDURE

Please read the instructions for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three up to five and the volume of Washing Buffer from 300 µL to 350 µL to avoid washing effects. Pay attention to chapter 11. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!
Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µL Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µL TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

7.1. Measurement

Adjust the ELISA microwell plate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA microwell plate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at **450 nm** and record the absorbance values for each standard/control and sample.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the **mean absorbance values** of all duplicates.

8. RESULTS

8.1. Run Validation Criteria

In order for an assay to be considered valid, these instructions for use have to be strictly followed and the following criteria must be met:

- **Substrate Blank:** Absorbance value < 0.100
- **Standard A:** Absorbance value < 0.200
- **Standard B:** Absorbance value < Standard C
- **Standard C:** Absorbance value < Standard D
- **Standard D:** Absorbance value < Standard E
- **Standard E:** Absorbance value < Standard F

Standard A < Standard B < Standard C < Standard D < Standard E < Standard F

If these criteria are not met, the test is not valid and must be repeated.

8.2. Calculation of Results

In order to obtain quantitative results of the 1+1000 Sample Dilution in ng/mL, plot the (mean) absorbance values of the 6 Standards A - F on (linear/linear) graph paper in a system of coordinates against their corresponding concentrations (0, 10, 16, 30, 50 and 75 ng/mL) and draw a standard calibration curve (absorbance values on the vertical y-axis, concentrations on the horizontal x-axis).

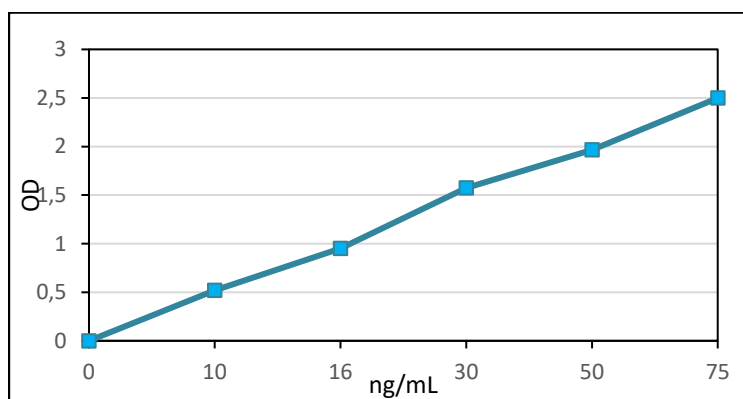
Read results from this standard curve employing the (mean) absorbance values of each sample.

For the calculation of the standard-curve mathematical Point to Point function should be used.

In order to calculate the final concentration of the undiluted Sample, the values from the calibration line have to be corrected by the sample dilution.

Example: The sample was diluted 1+1000 in Sample Dilution Buffer. The sample resulted in an OD of 0.950. According to the calibration line the concentration is e.g. 16.0 ng/mL. Now you have to multiply this with your dilution factor. In this example the factor is 1000. For this reason the final concentration in the sample is 16000 ng/mL respectively **16.0 µg/mL or 16.0 mg/L**.

8.3. Typical Standard Curve



8.4. Interpretation of Results and Recommendations - Preliminary-

Normal value ranges for this ELISA should be established by each laboratory based on its own sample populations in the geographical areas serviced.

The following values should be considered as a guideline:

Canine serum samples - Preliminary-		
Reference range Sample (1+1000) [ng/mL]	Rating	Clinical status
< 14	low	healthy
14-18	critical	notable value
> 18	medium-high	clinically evident
Diagnosis of a disease should not be established basically of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data.		

9. SPECIFIC PERFORMANCE CHARACTERISTICS

For further information about the specific performance characteristics please contact Gold Standard Diagnostics Frankfurt GmbH.

9.1. Precision

9.2. Analytical Sensitivity

The analytical sensitivity is defined as the apparent concentration of the analyte that can be distinguished from the zero calibrator.

9.3. Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin.

9.4. Cross Reactivity

9.5. Measurement range

10. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

11. PRECAUTIONS AND WARNINGS

- The test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the samples.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel who are familiar with good laboratory practice.
- For further internal quality control each laboratory should additionally use known samples.

11.1 Safety note for reagents containing hazardous substances

Reagents may contain CMIT/MIT (3:1) or MIT (refer to 3.1)

Therefore, the following hazard and precautionary statements apply.

Warning



H317	May cause an allergic skin reaction.
P261	Avoid breathing spray
P280	Wear protective gloves/protective clothing.
P302+P352	IF ON SKIN: Wash with plenty of soap and water.
P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
P362+P364	Take off contaminated and wash it before reuse.

Reagents may contain 5-Bromo-5-nitro-1,3-dioxane (refer to 3.1)

Therefore, the following hazard and precautionary statements apply.

Warning



H315	Causes skin irritation.
H319	Causes serious eye irritation.
P280	Wear protective gloves/ protective clothing.
P302+P352	IF ON SKIN: Wash with plenty of soap and water.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337+P313	If eye irritation persists: Get medical advice/attention.

Further information can be found in the safety data sheet.

11.2 Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

For information about the packaging materials refer to PACKAGING MATERIALS.

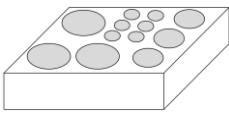
12. ORDERING INFORMATION

Prod. No.:	CCRPVT4050	VetLine Canine C-Reactive Protein (cCRP) ELISA (96 Determinations)
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ABBREVIATIONS

CMIT	5-chloro-2-methyl-4-isothiazolin-3-one
MIT	2-methyl-2H-isothiazol-3-one

PACKAGING MATERIALS

 PAP 21	 PAP 21	 PAP 22	 PAP 22
SOLN STOP WASH BUF 20x CONJS CAL SUB TMB DIL			MTP
 HDPE 2		 PP 5	
		 PET / ALU / LDPE 90	

SYMBOLS KEY

	Manufactured by
LOT	Lot Number
	Expiration Date
	Storage Temperature
REF	Catalogue Number
	Consult Instructions for Use
MTP	Microtiterplate
CONJS	Conjugate
CAL	Standard or Calibrator A-F
DIL	Sample Dilution Buffer
SOLN STOP	Stop Solution
SUB TMB	TMB Substrate Solution
WASH BUF 20x	"Washing Buffer (20x concentrated)"; REF W0000 Washing Buffer 20x concentrated
	Contains sufficient for "n" tests

SUMMARY OF TEST PROCEDURE

SCHEME OF THE ASSAY

VetLine Canine C-Reactive Protein (cCRP) ELISA

Test Preparation

Prepare reagents and samples as described.
Establish the distribution and identification plan for all samples and standards/controls.
Select the required number of microtiter strips or wells and insert them into the holder.

Assay Procedure

	Substrate Blank (A1)	Standard A - F	Sample (1+1000)
Standard A - F	-	100 µL	-
Sample (1+1000)	-	-	100 µL
Cover wells with foil supplied in the kit Incubate for 1 h at 37±1°C Wash each well three times with 300 µL of Washing Buffer			
Conjugate	-	100 µL	100 µL
Incubate for 30 min at room temperature (20...25 °C) Do not expose to direct sunlight Wash each well three times with 300 µL of Washing Buffer			
TMB Substrate Solution	100 µL	100 µL	100 µL
Incubate for exactly 15 min at room temperature (20...25 °C) in the dark			
Stop Solution	100 µL	100 µL	100 µL
Photometric measurement at 450 nm (reference wavelength: 620 nm)			



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