NovaTec Immundiagnostica GmbH

VetLine Canine C-Reactive Protein (cCRP)

ELISA RUO

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

Product Number: CCRPVT4050 (96 Determinations)

ENGLISH

1. INTRODUCTION

2. INTENDED USE

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

3. 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.

4. MATERIALS

4.1. 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.
- **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:	20	ng/mL; yellow cap
Standard E:	30	ng/mL; yellow cap
Standard F:	40	ng/mL; yellow cap

For hazard and precautionary statements see 12.1
For potential hazardous substances please check the safety data sheet.

4.1. Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)
- 1 Plate layout

4.2. 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

5. 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.

6. 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!

6.1. 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.

6.2. 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.

6.3. 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.

7. 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.

7.1. 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.

8. ASSAY PROCEDURE

Please read the instruction 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 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. 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.

8.1. 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 in the plate layout.

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

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

9. RESULTS

9.1. Run Validation Criteria

In order for an assay to be considered valid, 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 F:** Absorbance value **> 1.500**

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.

9.2. 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, 20, 30 and 40 ng/mL) and draw a standard calibration curve (absorbance values on the vertical y-axis, concentrations on the horizontal x-axis).

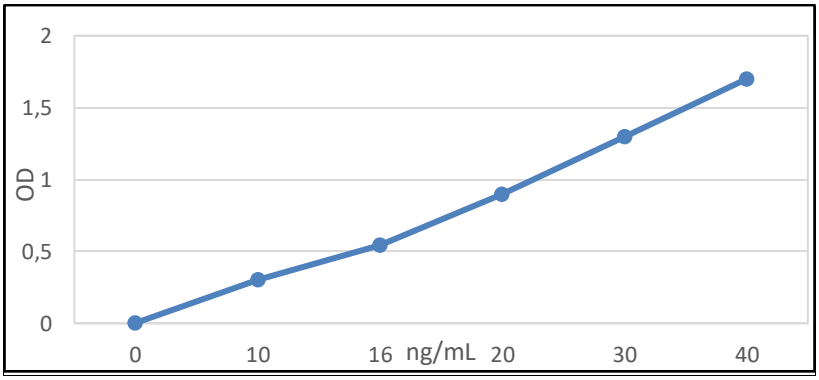
Read results from this standard curve employing the (mean) absorbance values of each sample.

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 0.900. According to the calibration line the concentration is e.g. 20.0 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 20000 ng/mL respectively **20.0 µg/mL or 20.0 mg/L.**

9.3. Typical Standard Curve



9.4. 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.		

10. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.

10.1. Precision

10.2. Analytical Sensitivity

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

10.3. 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.

10.4. Cross Reactivity

10.5. Measurement range

11. LIMITATIONS OF THE PROCEDURE

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

12. PRECAUTIONS AND WARNINGS

- **For research use only!**
- 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.

12.1 Safety note for reagents containing hazardous substances

Reagents may contain CMIT/MIT (3:1) or MIT (refer to 4.1)
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.

Further information can be found in the safety data sheet.

12.2. 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.

13. ORDERING INFORMATION

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

BIBLIOGRAPHY / LITERATUR / BIBLIOGRAPHIE / BIBLIOGRAFIA / BIBLIOGRAFÍA/ BLIBIOGRAFIA

ABBREVIATIONS / ABKÜRZUNGEN / ABRÉVIATIONS / ABBREVIAZIONI / ABREVIACIÓNES / ABREVIATURAS

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

SYMBOLS KEY / SYMBOLSCHLÜSSEL / EXPLICATION DES SYMBOLES / LEGENDA / SIMBOLOS / TABELA DE SIMBOLOS

	Manufactured by / Hergestellt von / Fabriqué par / Prodotto da / Fabricado por / Fabricado por
LOT	Lot Number / Chargenbezeichnung / Numéro de lot / Lotto / Número de lote / Número de lote
	Expiration Date / Verfallsdatum / Date de péremption / Scadenza / Fecha de caducidad / Data de Validade
	Storage Temperature / Lagertemperatur / Température de conservation / Temperatura di conservazione / Temperatura de almacenamiento / Temperatura de Armazenamento
RUO	For research use only / Nur für Forschungszwecke / Destiné à la recherche uniquement/ Solo per scopi di ricerca/ Uso exclusivo en investigación / Apenas para fins de pesquisa
REF	Catalogue Number / Katalog Nummer / Référence du catalogue / Numero di codice / Número de Catálogo / Número de Catálogo
	Consult Instructions for Use / Arbeitsanleitung beachten / Consulter la notice d'utilisation / Consultare le istruzioni per l'uso/ Consulte las Instrucciones de Uso / Consultar as Instruções de Utilização
MTP	Microtiterplate / Mikrotiterplatte / Plaque de Microtitrage / Piastre di Microtitolazione / Placa de Microtitulación / Placa de Microtitulação
CONJS	Conjugate / Konjugat / Conjugué / Coniugato / Conjugado / Conjugado
CAL	Standard or Calibrator A-F / Standard oder Kalibrator A-F / Standard o Etalon A-F / Standard o Calibratore A-F / Estándar o Calibrador A-F/ Standard ou Calibrador A-F
DIL	Sample Dilution Buffer / Probenverdünnungspuffer / Tampon de Dilution d'Échantillon / Tampone di Diluizione del Campione / Tampón de Dilución de Muestras / Tampão de Diluição de Amostra
SOLN | STOP	Stop Solution / Stopplösung / Solution d'Arrêt / Soluzione Bloccante / Solución de Parada /Solução de Bloqueio
SUB | TMB	TMB Substrate Solution / TMB-Substratlösung / Solution de Substrat TMB / Soluzione Substrato TMB / Solución de Sustrato de TMB / Solução Substrato TMB
WASH | BUF | 20x	Washing Buffer 20x concentrated / Waschpuffer 20x konzentriert / Tampon de Lavage concentré 20 x / Tampone di Lavaggio concentrazione x20 / Tampón de Lavado concentrado x20 / Tampão de Lavagem concentrada 20x
	Contains sufficient for "n" tests / Ausreichend für "n" Tests / Contenu suffisant pour "n" tests / Contenuto sufficiente per "n" saggi / Contenido suficiente para "n" tests / Conteúdo suficiente para "n" testes

SUMMARY OF TEST PROCEDURE / KURZANLEITUNG TESTDURCHFÜHRUNG / RÉSUMÉ DE LA PROCEDURE DE TEST / SCHEMA DELLA PROCEDURA / RESUMEN DE LA TÉCNICA / RESUMO DO PROCEDIMENTO DE TESTE

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 on the plate layout supplied in the kit.
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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