

LifeAssays[®] Canine CRP Test Kit

E-25-0002-09

Intended Use

LifeAssays[®] Canine CRP Test Kit is an in vitro veterinary diagnostic test for the quantitative determination of Canine C-Reactive Protein (CRP) in serum or plasma. It is intended to be used as an aid in the diagnosis, progression and treatment of various infections in dogs. For professional use only.

Content Information

Reagent Vial (Art.No. 40301-11/01)	10/20 pcs
<i>Disposable plastic vial containing reagent solution (0.75µL, 0.1% NaN₃)</i>	
Canine CRP Liquid Control (50µL, Art.No. 40201-03)	1 pcs
Glass Capillaries (5µL, Art.No. 40301-03)	25 pcs
Algorithm Chip (Art.No. 50501-01)	1 pcs
Instructions for Use Leaflet (E-25-0002)	1 pcs

Material Required but Not Supplied with Kit

- LifeAssays[®] VetReader (Art.No. 50101-04)
- Vortex e.g. LifeAssays[®] Vortex (Art.No. 50201-04)
- Capillary Holder (Art.No. 40301-04)
- LifeAssays[®] Dilution Kit (Art.No. 40301-07)
- LifeAssays[®] Canine CRP L.C. Kit (Art.No. 40201-07)

Stability and Storage

Store reagent kit and all components at 2–8° C. The reagent kit is stable until expiry date printed on the box. Avoid direct sunlight or exposure to temperatures above 25°C. Do not freeze.

Test Principle

LifeAssays[®] Canine CRP Test is a two-site heterogenous immunoassay. When the sample is placed into the reagent vial and the contents are mixed, polyclonal antibody coated silica microparticles, used as the solid phase in the assay, capture the CRP present in the sample. Polyclonal antibody coated magnetic nanoparticles detect the CRP bound to the silica surfaces in a sandwich-binding format. The reagent vial is then loaded into the LifeAssays[®] VetReader and the instrument waits eleven minutes before measuring the test result. During these eleven minutes the silica microparticles sediment to the bottom of the reagent vial forming a solid phase pellet. The magnetic property of the solid phase pellet is measured quantitatively with the LifeAssays[®] VetReader by detecting the inductance change of the internal coil.

Each LifeAssays[®] Canine CRP Test Kit is delivered with one disposable algorithm chip. The chip contains all reagent data and a self-executable algorithm which will calculate the CRP test result in mg/L for the number of purchased tests. When the chip calculates a CRP result, it transfers the result back to the LifeAssays[®] VetReader for display.

Warnings and Precautions

- For in vitro veterinary diagnostic use only.
- Do not mix components from different kit containers.
- Do not use test kits after the expiry date.
- Do not use damaged or contaminated kits.
- Bring all test kit components to room temperature (18–25 °C) before use.
- The reagents and canine CRP liquid control contain sodium azide in concentrations <0.1% as a preservative. Sodium azide is a toxic agent. Avoid contact with skin and eyes. Flush abundantly with water upon disposal or if direct contact occurs.
- Wear disposable gloves while handling samples, kit reagents or canine CRP liquid control, and wash hands thoroughly afterwards.
- The canine CRP liquid control contains serum from canine origin. Handle it with care as if capable of transmitting infectious agents.
- Disposal of all specimen and test material should be in accordance with state and local law.

Specimen Collection and Preparation

LifeAssays[®] Canine CRP test can be performed with plasma or serum samples. Heparin or granule activator can be used as anticoagulants.

Serum

A normal venous blood sample should be taken and the serum separated. If not immediately tested, the serum can be stored at 2–8 °C for two days or at -20 °C for one month.

Plasma

Collect a venous whole blood sample into a tube containing heparin as the anticoagulant. Separate the plasma from the blood cells immediately to avoid haemolysis. Mild haemolysis does not affect the test result. If not immediately tested, the plasma can be stored at 2–8 °C for two days or at -20 °C for one month.

Note: Repeated freezing and thawing of samples should be avoided. All samples must reach room temperature (18–25 °C) before testing. Frozen samples must be thawed completely, mixed thoroughly and brought to room temperature before testing is carried out.

Interferences

Heparin or granule activator used as anticoagulants do not interfere with the test. Increased levels of leukocytes, lipids, or bilirubin do not interfere with the assay performance.

Assay Procedure

Important Procedural Notes!

- Reagent vials and kit components must be allowed to reach room temperature (18–25 °C) before use.
- Always use a new and unbroken capillary. Avoid handling the capillary directly with your hands and without gloves (use capillary holder only).
- Reagent vials with reagent caught in the vial cap due to transport handling should be shaken (remove reagent from cap) and allowed to sediment for 15 minutes prior to use. To avoid loss of reagent never open a vial that has reagent caught in the cap.
- The procedure steps should be performed successively without any interruptions.
- Caps should be screwed on or closed tightly after use.
- For more information, see VetReader operator's manual.

1. Turn on Instrument

Insert the disposable algorithm chip provided with the LifeAssays[®] Canine CRP Test Kit and turn on the LifeAssays[®] VetReader. The instrument performs a reagent chip control and displays, "wait 113" (with a numerical countdown from 113). The instrument will stabilize for about 10 minutes and display during this time, "Stab T".

2. Start-up

Remove a reagent vial from storage and allow it to reach room temperature. It is recommended to remove the reagent vial at least 15 minutes before a measurement is performed. Reagent vials with reagent caught in the vial cap due to transport handling should be shaken (remove reagent from cap) and allowed to sediment for 15 minutes prior to use. Never open a reagent vial that has reagent caught in the cap. This could lead to loss of reagent and erroneous results.

Follow the instructions given on the display of the VetReader. Once stabilization is complete, the instrument is ready for use and will display, "Canine CRP Tests XX". To start a measurement press and hold the enter button. The instrument will display, "wait" (3-second pause) followed by "Insert vial". Load a reagent vial into the VetReader. The instrument will display, "wait 5" and count down 5 seconds before displaying "Collect + Add Sample".

3. Collect Sample

“Collect + Add Sample” will be displayed on the VetReader. Collect a 5µL serum or plasma sample using the glass capillary provided with the kit.

Note: Use the capillary holder (sold as an accessory) for capillary handling. Avoid the introduction of air bubbles into the glass capillary. Be sure to fill the entire length of the capillary with serum or plasma. Avoid excess sample on the outside of the capillary by “wiping off” excess with a clean tissue.

4. Add Sample

Unload the reagent vial from the VetReader. Open the cap of the reagent vial. Drop the capillary into the reagent vial and close the cap.

Note: The VetReader allows 5 seconds for adding sample before automatically switching to vortexing mode.

5. Mix using Vortex

“Vortex 30” will be displayed on the VetReader. Vortex the reagent vial for 30 seconds using the timer on display. Once the countdown has reached “0” the instrument will display, “Vortex OK”.

Note: Make sure the pellet is completely suspended after vortexing by turning the reagent vial upside down.

6. Loading of Reagent Vial into Instrument

“Insert Vial 10” will be displayed on the VetReader with a numerical countdown from 10 seconds. Load the reagent vial back into the VetReader before the display shows “0”. The instrument will display “Wait 660” and count down from 660 seconds (11 minutes). The user is now free to leave the instrument!

Note: The reagent vial must be loaded into the VetReader within 10 seconds after vortexing is completed. Otherwise, the measurement is aborted and the reagent chip will count down 1 measurement (the user loses one test).

7. Measurement

After 11 minutes the instrument will automatically perform 5 measurements, by moving the reagent vial in and out of the internal coil. The result is shown on the display in mg/L and will remain on the display until the reagent vial is unloaded from the instrument. The display on the instrument will show, “Canine CRP XX mg/L” where XX indicates a numerical value.

Note: The user should never press or push the reagent vial back into the instrument during movement. Such an action could damage the mechanical moving parts of the instrument.

8. Starting a New Measurement

Unload the reagent vial from the instrument. The display will show, “Canine CRP Test XX” where XX indicates the number of tests left on the reagent chip. To begin a new measurement press and hold the enter button. The instrument will display, “wait” (3-second pause) followed by “Insert vial”. Repeat the assay procedure from this point outlined in step 2.

Quality Control

The included Canine CRP Liquid Control (Art. No. 40201-03) or the accessory; Canine CRP Liquid Control Kit (Art. No. 40201-07) should be used to confirm the efficacy of the reagents and correct performance of the test. It is recommended to measure the control every time a new LifeAssays® Canine CRP Test Kit is opened. Routine measurement of the control allows for analysis of values and trends. Test the control according to the same procedure as for a canine sample.

References

- Caspi, D., F. W. J. J. Snel, R. M. Batt, D. Bennett, G. R. Rutteman, E. G. Hartman, M. L. Baltz, E. Gruys and M. B. Pepys, 1987: C-reactive protein in dogs. American Journal of Veterinary Research 48, 919–921.
- Yamamoto, S., T. Shida, T. Okimura, K. Otabe, M. Honda, Y. Ashinda, E. Furukawa, M. Sarikaputi and M. Naiki, 1994b: Determination of C-reactive protein in serum and plasma from healthy dogs and dogs with pneumonia by ELISA and slide reversed passive latex agglutination test. Veterinary Quarterly 16, 74–77.
- Kjelgaard-Hansen, M., A.L. Jensen and A.T. Kristensen, 2003: Evaluation of a Commercially Available Human C-Reactive Protein (CRP) Turbidometric Immunoassay for Determination of Canine Serum CRP Concentration. Veterinary Clinical Pathology 32, 81–87.

Further Reading:

Commissioned Research Report: Method evaluation and method comparison of LifeAssays canine CRP point-of-care system. M. Kjelgaard-Hansen, 2010. <http://www.lifeassays.com/files/documents/cre.pdf>

Demonstration Video

For a complete demonstration of LifeAssays® Canine CRP Test system, please scan the QR-code below with your smartphone.



Performance Characteristics

Analytical specificity

Polyclonal antibodies specific to canine CRP are used in the test. No other canine blood components are known to cross react with CRP in the LifeAssays® Canine CRP Test system.

Standardization

No international reference standard for Canine CRP is currently available. LifeAssays® Canine CRP is calibrated internally.

Measuring Range

Serum and plasma samples: 10–210 mg/L. For >210 mg/L results, samples may be diluted with PBS buffer and re-measured for a numerical result. Note: to calculate the final canine CRP concentration, the measured result must be multiplied by the dilution factor. For easy dilution handling use LifeAssays® Dilution Kit.

Reference Range

The reference range for CRP in serum is usually reported as less than 20 µg/L (Ref. 1–3). We recommend: that CRP serum or plasma concentrations ≥ 35 mg/L should be seen as a strong indication for systemic inflammation; and concentrations ≤ 10 mg/L as a strong indication for absence of systemic inflammation.

Detection Limit:

< 10 mg/L

Precision and Reproducibility

Intra-assay precision (n=4)			Inter-assay precision (n=8)		
CRP control, Mean (mg/L) CV%			CRP control, Mean (mg/L) CV%		
102	130	7.3	102	131	5.9
60	62	3.9	60	60	4.0
30	32	6.3	30	31	8.4
CRP control, Mean (mg/L) CV%			CRP control, Mean (mg/L) CV%		
40	27	10.9	40	27	9.5
Diluted	307	16.4	Diluted	334	16.7
Diluted	116	18.3	Diluted	109	17.1