



eurisTest™

Immunoturbidimetric Assay for Canine C-Reactive Protein (CRP)

Instruction For Use (IFU) manual- Version 3, December 2017

A quantitative detection of CRP in canine (dog) plasma or serum for automated clinical analyzers.

FOR VETERINARY AND RESEARCH USE ONLY.

1 INTENDED USE

The canine CRP eurisTest™ is an immunoturbidimetric immunoassay for the quantitative, *in vitro* determination of CRP in dogs, which can be a useful tool for monitoring systemic inflammation.

Art No.

2510-01	R1 assay buffer	2x 20 ml
	R2 antibody reagent	1x 8.0 ml
	CRP CAL200 calibrator	1x 0.5 ml
	CRP level 2 control	1x 0.5 ml

2 GENERAL DESCRIPTION¹⁻⁴

CRP is a pentameric serum protein that consists of five 20 kDa subunits (of which two are glycosylated). The protein is a well-known acute phase reactant and its normal plasma concentrations in healthy dogs is <35 mg/l. CRP is a real-time diagnostic marker for systemic inflammation with plasma concentrations increasing approximately 4 hrs after stimulation, peaking around 24 hrs and clearing between 48-72 hrs after cessation of inflammatory conditions. Measurement of CRP has a large diagnostic window, increasing more than 10x the normal plasma concentrations during inflammatory activity. Clinical use of CRP is not limited to monitoring systemic inflammation, but can also be used for efficacy of selected treatment or monitoring post-operative conditions and surgery recovery.

3 ASSAY PRINCIPLE

The canine CRP eurisTest™ is a quantitative immunoturbidimetric immunoassay for the detection of CRP in canine (dog) plasma or serum. The R2 antibody reagent contains polyclonal antibodies against canine CRP. Upon mixing of reagents, the CRP antigen present in the canine sample together with the R2 antibody reagent forms a precipitation reaction which yields a turbid solution. The turbidity of the solution is measured photometrically at 340 nm and is directly proportional to the concentration of CRP present in the canine sample.

4 COMPOSITION OF SUPPLIED REAGENTS

Contents	Substance & Concentration
R1 assay buffer (1502-16)	max 4% polyethylene glycol max 50 mM Tris buffer, pH 7.6 150 mM NaCl
R2 antibody reagent (1502-14)	goat anti-canine CRP serum
CRP CAL200 calibrator (1502-17)	200 µg/ml
CRP level 2 control (1502-45)	60 + 15 µg CRP/ml
Instruction For Use (IFU) (1810-02)	1 copy for laboratory

5 MATERIALS NEEDED BUT NOT SUPPLIED

- Sample collection device and/or pipette
- Disposable gloves
- Timer
- NaCl solution, 0.9 % (w/v)

6 STORAGE & STABILITY

The R1 assay buffer, R2 antibody reagent, canine CRP CAL200 calibrator, and the canine CRP level 2 control are supplied ready-to-use and are stable up to the expiry date when stored at +2-8 °C. They may not be frozen. Screw caps carefully after use of controls and kit reagents to avoid evaporation.

7 PRECAUTIONS

- FOR VETERINARY AND RESEARCH USE ONLY.
- Do not use after expiration date.
- Do not freeze any test reagents.
- Lipaemia, haemolytic samples or high levels of detergents in sample may interfere with assay results.
- Follow Good Laboratory Practices, wear a lab coat, use disposable gloves and keep laboratory area clean.
- Reagents and controls are from animal origin and should always be handled with due caution.
- After use, the test should be discarded according to local regulations regarding biological and hazardous material.
- Avoid evaporation of reagents and controls.

8 SAFETY & WASTE HANDLING

Only qualified laboratory personnel under appropriate laboratory conditions may use the reagents. CAUTION: kit components contain sodium azide (<0.1%) as preservative. Therefore, handle as hazardous material and wear disposable gloves, eye protection and a lab coat. Do not ingest! Avoid contact with skin, mucous membranes and eyes. If uncertain, consult expertise for help. Health and Data Sheets are available at request. Handling of waste should be done in accordance with national laws and local regulations.

9 SPECIMEN COLLECTION

Collect canine (dog) lithium heparin plasma or serum sample using a blood collection tube according to the manufacturer's instructions. Do not use EDTA collection tubes. The stability of canine CRP serum is 2 weeks at +2-8 °C. For long-term storage, the specimen must be kept frozen (<-20°C). Repetitive freezing and thawing cycles is not recommended. The sample must be completely thawed, thoroughly mixed and at room temperature before testing can occur.

10 INSTRUMENT PARAMETERS

Recommended setting of parameters for automatic analyzers are:

- 340 nm
- 1 cm light path
- Reaction temperature: +37 Celsius
- Reagent blank as reference
- Volume R1: 200 µl
- Volume S (sample): 15 µl
- Volume R2: 40 µl
- Reaction time 1 (R1+S): 5 min
- Reaction time 2 (R1+S+R2): 5 min

11 PROCEDURE

Mix 200 µl R1 assay buffer with 15 µl Sample (S) and let incubate for 5 minutes at 37 °C. Read the Absorbance (A1). After addition of 40 µl R2 antibody reagent mix and incubate for 5 minutes at 37 °C. Read the Absorbance (A2). Calculate $\Delta A = A2 - A1$. The sample (S) is either water (blank), dog serum/plasma or calibrator (canine CRP CAL 200 or diluted with 0.9 % (w/v) NaCl solution to 100, 50, 25 and 12.5 µg canine CRP/ml). The recommendation is to use multi-point calibration.

12 CALIBRATION & QUALITY CONTROL

Use the canine CRP CAL200 Calibrator (Art. No. 1502-17) supplied with the kit for calibration of the assay. A spline or logit/log based calculation is required. Calibration should be repeated when required by Quality Control or with each new kit.

In order to survey accuracy and precision, daily quality control is recommended with canine CRP Level 2 Control (Art. No. 1502-45). If the value of the control falls outside the acceptable measuring limit and repetition of measurement does not change control result, please check:

1. Instrument settings and light source.
2. Overall cleanliness of equipment in use.
3. Solutions used, including water. Contaminants, such as, bacterial growth may yield inaccurate results.
4. Reaction temperature.
5. Expiry date of kit and contents.

After completing check list, if the control value still falls outside the acceptable limits, contact European Institute of Science technical support: info@euris.org.

13 PERFORMANCE

Assay Measuring Range: The measuring range of the assay is 10 – 300 mg/L (10 – 300 µg/mL). Samples with canine CRP levels larger than 200 mg/L (200 µg/mL) should be diluted 1:4 with 0.9 % (w/v) NaCl solution and the result multiplied with 4.

Sensitivity: The minimum level of detection is approximately 5 mg/L (5 µg/mL).

Prozone limit: No prozone effect can be observed for canine CRP concentrations of up to 1 500 mg/L (1 500 µg/mL).

Specificity & Interference: The antiserum used is monospecific for canine CRP. It has not been shown to cross-react with other serum proteins under the conditions of the assay. However, the assay may be interfered by Lipaemina, Haemolytic samples or high levels of detergents in sample.

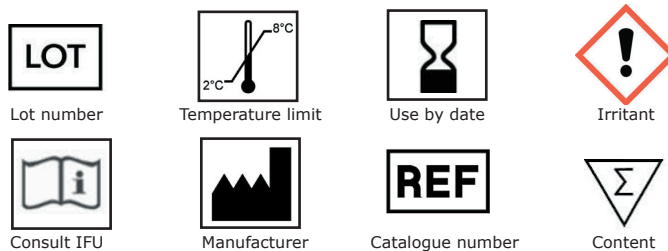
Precision: The precisions of the assay is <5%.

Normal Ranges: The normal range of the CRP concentration in healthy dogs is <35 mg/L (35 µg/mL). Each laboratory should establish its own normal range which corresponds to local genetic and environmental factors.

• Repetitive measurement of canine CRP can be used to determine if selective treatment is effective and for the monitoring of post-operative conditions and surgery recovery.

• Canine CRP results should be used with other clinical and diagnostic information for forming a diagnosis and for health management.

14 SYMBOLS KEY



15 REFERENCES

[1] Ganrot K., Plasma protein respons in experimental inflammation in dogs., Res. Exp. Med., 1973, 161(4), 251-261.

[2] Hansson L.O., Lindquist L. C-Reactive protein: its role in the diagnosis and follow-up of infectious diseases. Curr. Opin. Infect. Diseases, 1997, 10:196-201.

[3] Yamamoto S., Changes in serum C-reactive protein levels in dogs with various disorders and surgical traumas, Vet. Res. Com. 1993, 17:85-93.

[4] Kjelgaard-Hansen M., Lundorff Jensen A.T., Evaluation of a commercially available Human C-Reactive Protein (CRP) turbidimetric immunoassay for determination of Canine Serum CRP concentration, Vet Clin Pathology, 2003, 32:2, 81-84.

Manufactured by: European Institute of Science AB

