Canine CRP Detection Kit

Catalog # 6027

For Research Use Only - Not Human or Therapeutic Use

Acute phase proteins (APPs) are blood proteins released from hepatocytes as an integral part of acute phase responses (APR). The APR, as a part of the innate host defense system, is triggered by tissue damage and inflammation caused by infectious, immunologic, or neoplastic agents. One of the well-characterized APPs, C-reactive protein (CRP) is an annular pentameric protein consisting of 224 amino acids (25kDa) (1). CRP binds to the phosphocholine expressed on the surface of dead cells and bacteria (2). These complexes activate the complement system, leading to the phagocytic removal of the complexes by macrophages.

CRP levels in canine serum are significantly elevated after inflammatory irritation, surgical trauma, or inflammatory diseases such as pyometra, panniculitis, acute pancreatitis, polyarthritis, septic arthritis, and hemangiosarcoma (3-7). Therefore, serum CRP is considered a measure of inflammation in canines. Interestingly, CRP is also found in canine saliva, and saliva CRP levels correlate with serum CRP levels. Because blood collection is an invasive procedure, saliva collection presents an alternative method for CRP collection. Like serum CRP, saliva CRP can be used as a marker to monitor inflammation status (8). However, as saliva CRP levels are about 1% of serum CRP levels, a highly sensitive and reliable assay is required (5, 9-11).

Chondrex, Inc provides a canine CRP ELISA kit which is compatible with both serum and saliva samples. It can be used for detecting and monitoring canine inflammation, as well as in studies investigating inflammation. An immunochromatographic test for veterinary use is currently in development. Please contact Chondrex, Inc. at support@chondrex.com for more information.

KIT COMPONENTS

Item	Quantity	Amount	Storage
Canine CRP Standard (60271)	1 vial	100 ng, lyophilized	-20°C
Canine CRP Detection Antibody (60273)	1 vial	50 µl	-20°C
Solution B - Sample/Standard/Detection Antibody Dilution Buffer (67015)	1 bottle	50 ml	-20°C
TMB (90023)	2 vials	0.2 ml	-20°C
Chromogen Dilution Buffer (90022)	1 bottle	20 ml	-20°C
Stop Solution - 2N Sulfuric Acid (9016)	1 bottle	10 ml	-20°C
Wash Buffer, 20X (9005)	1 bottle	50 ml	-20°C
Capture Antibody Coated 96-Well ELISA Plate	1 each	8-well strips x 12	-20°C

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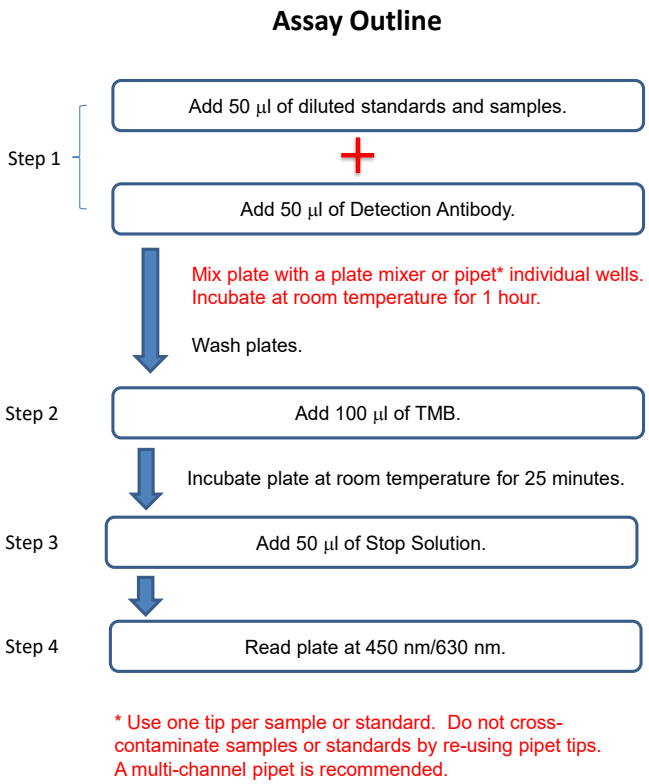
ASSAY OUTLINE



* Use one tip per sample or standard. Do not cross-contaminate samples or standards by re-using pipet tips. A multi-channel pipet is recommended.

NOTES BEFORE USING ASSAY

Note 1: It is recommended that the standard and samples be run in duplicate.

Note 2: Warm up all buffers to room temperature before use.

Note 3: Any partially used reagents may be kept in their original containers and stored at –20°C for future use.

Note 4: Crystals may form in Wash Buffer, 20X when stored at cold temperatures. If crystals have formed, warm the wash buffer by placing the bottle in warm water until crystals are dissolved completely.

Note 5: Measure exact volume of buffers using a serological pipet, as extra buffer is provided.

Note 6: Cover the plate with plastic wrap or a plate sealer after each step to prevent evaporation from the outside wells of the plate.

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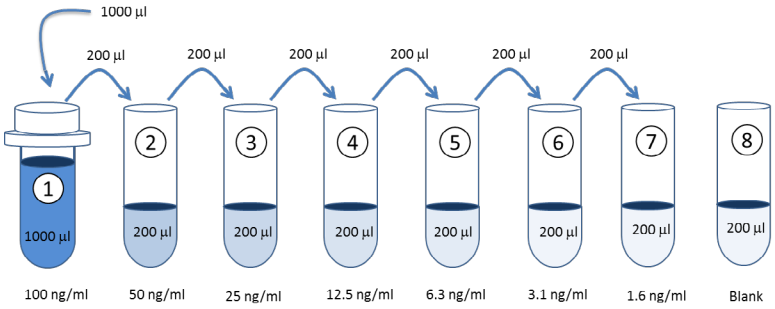
ASSAY PROCEDURE

1. **Prepare Standard Dilutions**: The recommended standard range is 1.6-100 ng/ml. Dissolve one vial of standard in 1 ml of Sample/Standard/Detection Antibody Dilution Buffer (Solution B) for the 100 ng/ml standard. Then serially dilute it with Solution B. For example, mix 200 µl of the standard (100 ng/ml) with an equal volume of Solution B to make a 50 ng/ml solution, and then repeat it five more times for 25, 12.5, 6.3, 3.1, and 1.6 ng/ml solutions. The remaining 100 ng/ml standard stock may be stored at -20°C for use in a second assay. We recommend making fresh serial dilutions for each assay.



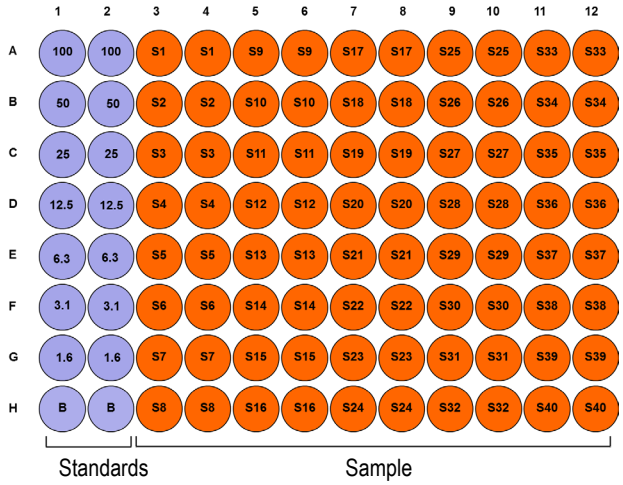
2. **Prepare Sample Dilutions**: Centrifuge samples at 10,000 rpm at 4°C for 3 minutes to remove insoluble materials and lipids. At minimum, dilute the samples with an equal volume of Solution B. For example, take 100 µl of a sample, and mix with 100 µl of Solution B. If the CRP level is higher than 100 ng/ml, re-assay the samples at a higher dilution.

3. **Prepare Detection Antibody**: Prepare detection antibody solution with Sample/Standard/Detection Antibody Dilution Buffer (Solution B) as shown in the following table.

Strip #	Detection Antibody (µl)	Solution B (ml)
2	8	0.9
4	17	1.7
6	25	2.5
8	33	3.3
10	41	4.1
12	50	5.0

4. **Add Standards and Samples**: Add Standards, Samples, and Detection Antibody: Mix standards, samples, and detection antibody tubes well. Add 50 µl of Solution B (blank), standards, and samples to appropriate wells (Figure 1). Add 50 µl of diluted detection antibody solution to all wells. Mix all wells by pipetting or use a plate shaker. Cover the plate with a plate sealer and incubate at room temperature for 1 hour.

Figure 1 - A Standard Assay Layout



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5. **Wash**: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blot on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

6. **Add TMB Solution**: Use new tubes when preparing TMB solution. Just prior to use, prepare TMB solution with Chromogen Dilution Buffer as shown in the following table.

Strip #	TMB (µl)	Chromogen Dilution Buffer (ml)
2	34	1.7
4	66	3.3
6	100	5.0
8	132	6.6
10	164	8.2
12	200	10.0

   Add 100 µl of TMB solution to each well immediately after washing the plate and incubate for 25 minutes at room temperature.

7. **Stop**: Stop the reaction with 50 µl of 2N Sulfuric Acid (Stop Solution) to each well.

8. **Read Plate**: Read the OD values at 450 nm. If the OD values of samples are greater than the OD values of the highest standard, re-assay the samples at a higher dilution. A 630 nm filter can be used as a reference.

CALCULATION OF RESULTS

1. Average the duplicate OD values for the blank, standards, and test samples.
2. Subtract the "blank" (B) values from the averaged OD values in step 1.
3. Plot the OD values of standards against the concentration of CRP (ng/ml). Using a log/log plot will linearize the data. Figure 2 shows a representative experiment where the standard range is 1.6-100 ng/ml.
4. The ng/ml of CRP in test samples can be calculated using regression analysis.

Figure 2 - A typical standard curve for CRP assay

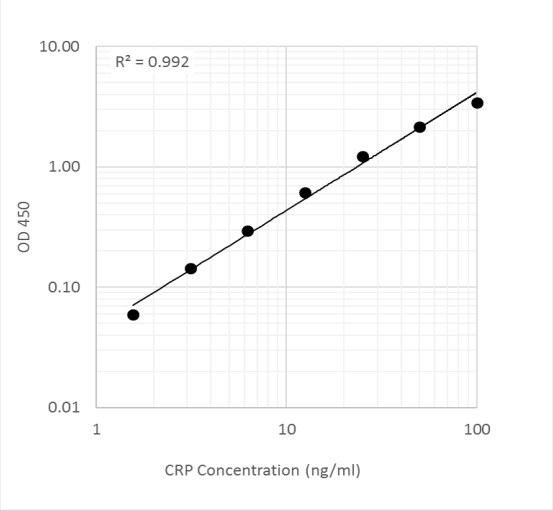

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Table 1 - Reproducibility for canine CRP ELISA Kit

Test	3.2 ng/ml	12.5 ng/ml	50 ng/ml
Inter-Assay CV (%)	6.0	9.0	8.7
Intra-Assay CV (%)	1.0	10.0	0.7
Spike Test*	108%	105%	109%

* Known amounts of canine CRP were added to samples and then diluted with Sample/Standard/Detection Antibody Dilution Buffer (Solution B).

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