Bovine C-Reactive Protein (CRP) ELISA

For the quantitative determination of C-Reactive Protein (CRP) in bovine serum and plasma

For Research Use Only. Not for use in Diagnostic Procedures.

Catalog Number: 41-CRPBO-E01
Size: 96 wells
Version: 4.1 - ALPCO 1.0

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1 INTENDED USE

The Bovine CRP ELISA is a highly sensitive two-site enzyme linked immunoassay (ELISA) for the determination of C-Reactive Protein (CRP) in bovine serum and plasma samples. For Research Use Only. Not for Use in Diagnostic Procedures.

2 PRINCIPLE OF THE TEST

The principle of the double antibody sandwich ELISA is represented in Figure 1. In this assay the C-Reactive Protein (CRP) present in samples reacts with the anti-CRP antibodies, which have been adsorbed to the surface of polystyrene microtiter wells. After the removal of unbound proteins by washing, anti-CRP antibodies conjugated with horseradish peroxidase (HRP) are added. These enzyme-labeled antibodies form complexes with the previously bound CRP. Following another washing step, the bound enzyme is assayed by the addition of a chromogenic substrate, 3,3',5,5'-tetramethylbenzidine (TMB). The quantity of bound enzyme varies directly with the concentration of CRP in the sample tested; thus, the absorbance, at 450 nm, is a measure of the concentration of CRP in the test sample. The quantity of CRP in the test sample can be interpolated from the standard curve constructed from the standards and corrected for sample dilution.

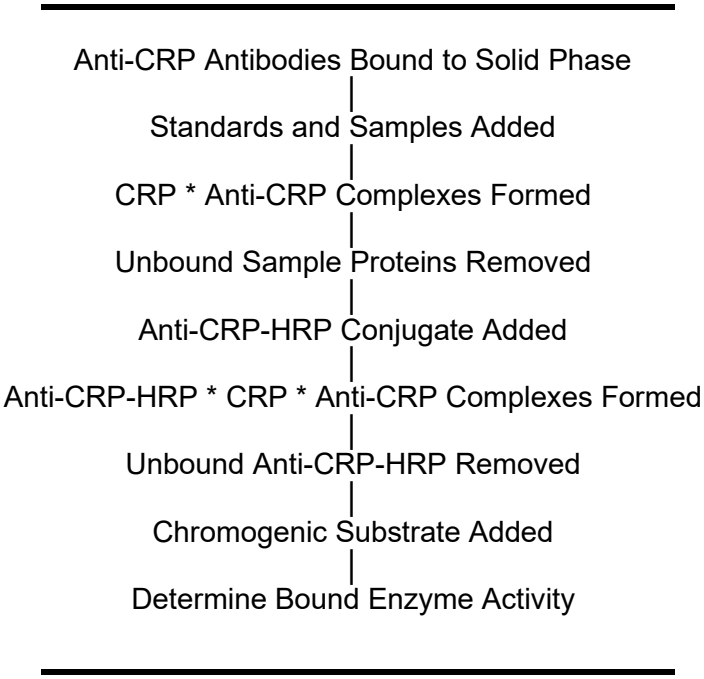


Figure 1

3 LIMITATIONS OF THE PROCEDURE

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Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the information contained in the package insert instructions and with adherence to good laboratory practice. Factors that might affect the performance of the assay include instrument function, cleanliness of glassware, quality of distilled or deionized water, and accuracy of reagent and sample pipetting, washing technique, incubation time or temperature. Do not mix or substitute reagents with those from other lots or sources.

4 MATERIALS PROVIDED

The expiration date for the kit and its components is stated on the box label. All components should be stable up to the expiration date if stored and used per this kit insert.

Component	Description	Preparation	Storage	Stability
ELISA Microplate, antibody coated	One plate of 12 removable 8 well strips, antibody coated	Ready to use as supplied.	2-8°C, In sealed foil bag with desiccant	With proper storage the plate strips are stable until the expiration date.
Enzyme Conjugated Detection Antibody	One vial of 150µL of 100X Horseradish Peroxidase Conjugated antibody in a stabilizing buffer	Dilute 1:100 immediately prior to use.	2-8°C in the dark	The 1X working conjugate should be diluted immediately prior to use. The 100X conjugate is stable until the expiration date.
Calibrator	One vial of calibrator	Refer to the Certificate of Analysis (CoA).	2-8°C for lyophilized calibrator. Aliquoted and frozen if re-constituted. Avoid multiple freeze-thaw cycles.	The 1X working standard solutions should be prepared immediately prior to use.
Diluent Concentrate	One 50 mL bottle of 5X diluent buffer	Dilute 1:5 to make 1X working solution.	2-8°C for both 1X working solution and 5X concentrate	The 1X working solution is stable for at least one week from the date of preparation. The 5X concentrate is stable until the expiration date.
Wash Solution Concentrate	One 50 mL bottle of 20X wash solution	Dilute 1:20 to make 1X working solution.	2-8°C for both 1X working solution and 20X concentrate	The 1X working solution is stable for at least one week from the date of preparation. The 20X concentrate is stable until the expiration date.
Chromogen-Substrate Solution	One bottle of 12 mL 3,3',5,5'-tetramethylbenzidine (TMB) and hydrogen peroxide in citric acid buffer at pH 3.3.	Ready to use as supplied	2-8°C in the dark	Protect from light. The Substrate Solution is stable until the expiration date.
STOP Solution **WARNING: Avoid Contact with Skin**	One 12 mL bottle of 0.3 M sulfuric acid.	Ready to use as supplied	2-8°C	The Stop Solution is stable until the expiration date.

5. MATERIALS REQUIRED BUT NOT PROVIDED

- Precision pipettes (2 µL to 100 µL) for making and dispensing dilutions
- Test tubes
- Squirt bottle or Microtiter washer/aspirator
- Distilled or deionized H_2O
- Microtiter plate reader
- Assorted glassware for the preparation of reagents and buffer solutions
- Centrifuge for sample collection
- Anticoagulant for plasma collection
- Timer

6. SAMPLE COLLECTION AND HANDLING

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions when handling and disposing.

If blood samples are clotted, grossly hemolyzed, lipemic, or the integrity of the sample is of concern, make a note and interpret results with caution.

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

- Serum samples - Blood should be collected by venipuncture. The serum should be separated from the cells after clot formation by centrifugation. Remove serum and assay immediately or aliquot and store samples at –80°C (preferably) or -20°C. Avoid repeated freeze-thaw cycles.

- Plasma samples - Blood should be collected into a container with an anticoagulant and then centrifuged. Assay immediately or aliquot and store samples at -80°C (preferably) or -20°C. Avoid repeated freeze-thaw cycles.

- Known interfering substances - Azide and thimerosal at concentrations higher than 0.1% inhibit the enzyme reaction.

7. SAMPLE DILUTION

The assay requires that each test sample be diluted before use. All samples should be assayed in duplicate each time the assay is performed. The recommended dilutions are only suggestions. Dilutions should be based on the expected concentration of the unknown sample such that the diluted sample falls within the dynamic range of the standard curve. If unsure of sample level, a serial dilution with one or two representative samples before running the entire plate is highly recommended.

- Serum and Plasma Samples – Recommended starting dilution is 1:80,000. To prepare a 1:80,000 dilution of a sample, transfer 2 µL of sample to 1,998 µL of 1X diluent. This yields a 1:1,000 dilution. Next, dilute the 1:1,000 by transferring 10 µL into 790 µL of 1X diluent. This yields a 1:80,000 dilution. Mix thoroughly at each stage.

8. REAGENT PREPARATION

- **Bring all reagents to room temperature (16°C to 25°C) before use.**

- Diluent Concentrate - The Diluent Solution supplied is a 5X Concentrate and must be diluted 1:5 with distilled or deionized water (1 part buffer concentrate, 4 parts dH_2O).

- Wash Solution Concentrate - The Wash Solution supplied is a 20X Concentrate and must be diluted 1:20 with distilled or deionized water (1 part buffer concentrate, 19 parts dH_2O). Crystal formation in the concentrate may occur when storage temperatures are low. Warming of the concentrate to 30-35°C before dilution can dissolve crystals.

- Enzyme-Antibody Conjugate - Calculate the required amount of working conjugate solution for each microtiter plate test strip by adding 10 µL Enzyme-Antibody Conjugate to 990 µL of 1X Diluent for each test strip to be used for testing. Dilute immediately before use and protect from light. Mix uniformly, but gently. Avoid foaming.

- Pre-coated ELISA Microplate - Ready to use as supplied. Unseal foil pouch and remove plate from pouch. Remove all strips and wells that will not be used in the assay and place back in pouch and re-seal along with desiccant.

- Bovine CRP Calibrator – Prepare according to the lot-specific Certificate of Analysis.

9. ASSAY PROCEDURE

1. **All samples and standards should be assayed in duplicate.**

2. The Standards and the test sample(s) should be loaded into the ELISA wells as quickly as possible to avoid a shift in OD readings. Using a multichannel pipette reduces this occurrence.

   Pipette 100 µL of

Standard 0	(0.0 ng/mL) in duplicate
Standard 1	(1.57 ng/mL) in duplicate
Standard 2	(3.13 ng/mL) in duplicate
Standard 3	(6.25 ng/mL) in duplicate
Standard 4	(12.5 ng/mL) in duplicate
Standard 5	(25 ng/mL) in duplicate
Standard 6	(50 ng/mL) in duplicate
Standard 7	(100 ng/mL) in duplicate

3. Pipette 100 µL of sample (in duplicate) into pre-designated wells.

4. Incubate the microtiter plate at room temperature for sixty (60 ± 2) minutes. Keep plate covered and level during incubation.

5. Following incubation, aspirate the contents of the wells.

6. Completely fill each well with 1X working Wash Solution and aspirate. Repeat three times, for a total of four washes. If washing manually: completely fill wells with 1X working wash solution, invert the plate then pour/shake out the contents in a waste container. Follow this by sharply striking the wells on absorbent paper to remove residual buffer. Repeat 3 times for a total of four washes.

7. Pipette 100 µL of 1X working Enzyme-Antibody Conjugate to each well. Incubate at room temperature for thirty (30 ± 2) minutes. Keep plate covered in the dark and level during incubation.

8. Wash and blot the wells as described in Steps 5/6.

9. Pipette 100 µL of TMB Substrate Solution into each well.

10. Incubate in the dark at room temperature for precisely ten (10) minutes.

11. After ten minutes, add 100 µL of Stop Solution to each well.

12. Determine the absorbance (450 nm) of the contents of each well within 30 minutes. Calibrate the plate reader to manufacturer's specifications.

10. CALCULATION OF RESULTS

1. Subtract the average background value (Average absorbance reading of Standard zero) from all values.

2. Average the duplicate readings for each standard and use the results to construct a Standard Curve. Construct the standard curve by reducing the data using computer software capable of generating a four parameter logistic curve fit. A second order polynomial (quadratic) or other curve fits may also be used; however, they will give a less precise fit of the data.

3. Interpolate test sample values from the standard curve. Correct for sera dilution factor to arrive at the CRP concentration in original samples.

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