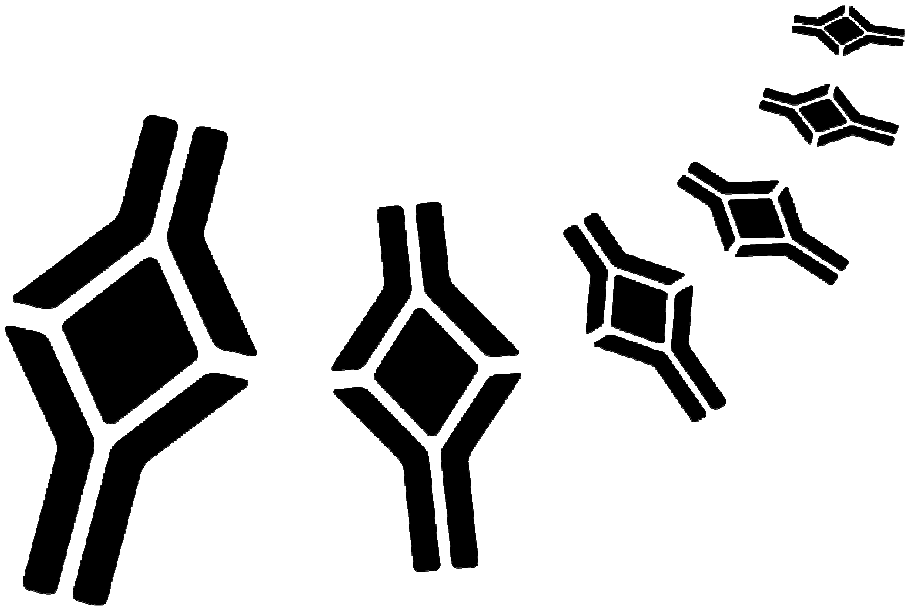
BioVendor
Research
and Diagnostic Products



Mouse C-Reactive Protein ELISA

Product Data Sheet

Cat. No.: RH971CRP01MR

For Research Use Only

VERSION 51 080908 18

CONTENTS

» **This kit is manufactured by:**
BioVendor – Laboratorní medicína, a.s.

» **Use only the current version of Product Data Sheet enclosed with the kit!**

1. INTENDED USE

The C-reactive protein assay is intended for the detection and quantification of mouse C-reactive protein (CRP) in mouse serum, plasma, and fluids. C-reactive protein is an acute-phase protein produced by the liver in conditions of inflammation, bacterial infection, or tissue trauma. Quantification of CRP is useful in determining inflammatory conditions difficult to diagnose and to monitor the patients' response to treatment.

2. CLINICAL RELEVANCE

C-reactive protein is synthesized in the liver following tissue damage caused by inflammation, infection, or trauma. It is considered to be an important acute phase marker in such conditions.

3. PRINCIPLE OF THE TEST

Mouse sera/plasma for testing are diluted to 1:20 (suggested) and allowed to react with pneumococcal C-polysaccharide coated on specially treated microwells. After appropriate incubation, the wells are washed to remove unreacted serum proteins, and an enzyme-labeled rabbit anti-CRP (conjugate) is then added to react with and tag the antigen-antibody complexes. Following another incubation period, the wells are again washed to remove unreacted conjugate. A urea peroxide substrate with TMB as chromogen is added to start color development. Development of a blue color indicates a positive reaction while negative reactions appear colorless or with a trace of blue. The reaction is interrupted with a stop solution that turns the blue positive reactions to yellow. Negative reactions remain colorless or with a hint of yellow. Color intensity (absorbance) is read at a wavelength of 450 nm on a spectrophotometer or ELISA reader. Semi-quantification of absorbance can be accomplished by the use of a standard curve generated by measuring two-fold dilutions of the standard provided.

4. MATERIALS SUPPLIED

The C-Reactive Protein kit supplies sufficient materials for 96 determinations.

1. **CRP ELISA microplate**
   96-well plate containing pneumococcal C-polysaccharide and packaged with desiccant, ready to use.
2. **Conjugate (100x) 0.13 mL**
   Concentrated affinity-purified horseradish peroxidase (HRP)-labeled rabbit anti-CRP-IgG with stabilizers and a preservative. Protect from light.
3. **CRP Standard, 2.5 µg/mL (100X), 0.13 ml.**
   Mouse serum with elevated CRP concentration. Serially dilute in doubling dilutions five times, diluting the provided Standard 1:100 for the first standard.
4. **Wash Buffer (1 packet)**
   Tris with Tween 20™, pH 7.4. Reconstitute to 1L with distilled water. Store at 2 – 8°C.
5. **TMB Substrate, 12 mL**
   A solution containing urea peroxide and 3,3', 5,5'-tetramethylbenzidine (TMB) supplied in a protective opaque bottle. Ready to use. Protect from light. Non-carcinogenic.
6. **Stop Solution, 12 mL** Diluted phosphoric acid. Ready to use.

5. MATERIAL REQUIRED BUT NOT SUPPLIED

1. Distilled or deionized (purified) water
2. Clean 250 or 500 mL wash bottle for wash buffer.
3. Test tubes or microtiter plate for preparing standard dilutions.
4. Precision pipette(s) (2 µL to 1000 µL) for making and delivering dilutions.
5. Adhesive cover for microplates.
6. ELISA reader equipped with a 450 nm filter. A program for data reduction would be helpful.

6. PREPARATION AND STORAGE OF REAGENTS

C-reactive protein kit components should be stored at 2-8°C. Bring them to room temperature (20-25°C) before opening bottles and plate pouches. Diluted conjugate remaining after use should be discarded. TMB substrate and stop solution are also stable at room temperature

7. PRECAUTIONS

1. DO NOT INTERCHANGE COMPONENTS BETWEEN KITS AND DIFFERENT LOTS OF THE SAME TEST.
2. The standard serum and conjugate have not been screened for infectious agents. Since no testing can assure the absence of infectious agents, however, these reagents, as well as the serum specimens and equipment coming in contact with these specimens, should be handled with good laboratory practices to avoid skin contact and ingestion.
3. Do not use components past expiration date.
4. HRP-labeled conjugate and TMB-substrate are photosensitive and are packaged in a protective opaque bottle. Store in the dark and return to storage after use.

8. SPECIMEN ABLE TO BE USED FOR THIS ASSAY

- Serum
- Plasma
- Urine
- Culture Supernatant
- Tissue Extracts
- Synovial Fluid

9. PREPARATION OF OTHER SPECIMEN TYPES

Samples other than blood (plasma or serum) should be used at a higher concentration. It is recommended to begin diluting the sample at 1:2 and increasing the dilution factor to values the user sees fit.

10. ASSAY PROCEDURE

PROCEDURAL NOTES

1. **IMPORTANT:**
   Bring kit components to room temperature (20-25°C) before opening bottles and plate pouches. Allow at least 30 minutes for this process.
2. MULTI-SAMPLE DILUTION:
   For assays on many samples, it is advisable to pre-dilute samples onto microtiter dilution wells before using a multichannel pipetter to transfer it onto the coated wells. This will ensure the least variable incubation period in samples in multiple test strips.
3. DUPLICATE/TRIPLICATE TESTING:
   It is highly recommended to run standards and unknown samples in duplicates or triplicates to ensure precision.

TEST PROCEDURE

1. Prepare wash buffer by adding 1 packet of powder to 1L of distilled water.
2. Prepare the standards as follows:
   - **Standard #1 = 25 ng/mL**: Dilute provided standard 1:100, e.g. 1 unit of standard plus 99 units of wash buffer.
   - **Standard #2 = 12.5 ng/mL**: Dilute Standard #1 two-fold, e.g. 1 unit of standard #1 plus 1 unit of wash buffer.
   - **Standards #3 (6.25 ng/mL)**, standard **#4 (3.13 ng/mL)**, and **standard #5** are prepared by serial two-fold dilutions following **standard #2**.

Please consider the following dilution scheme as a guide

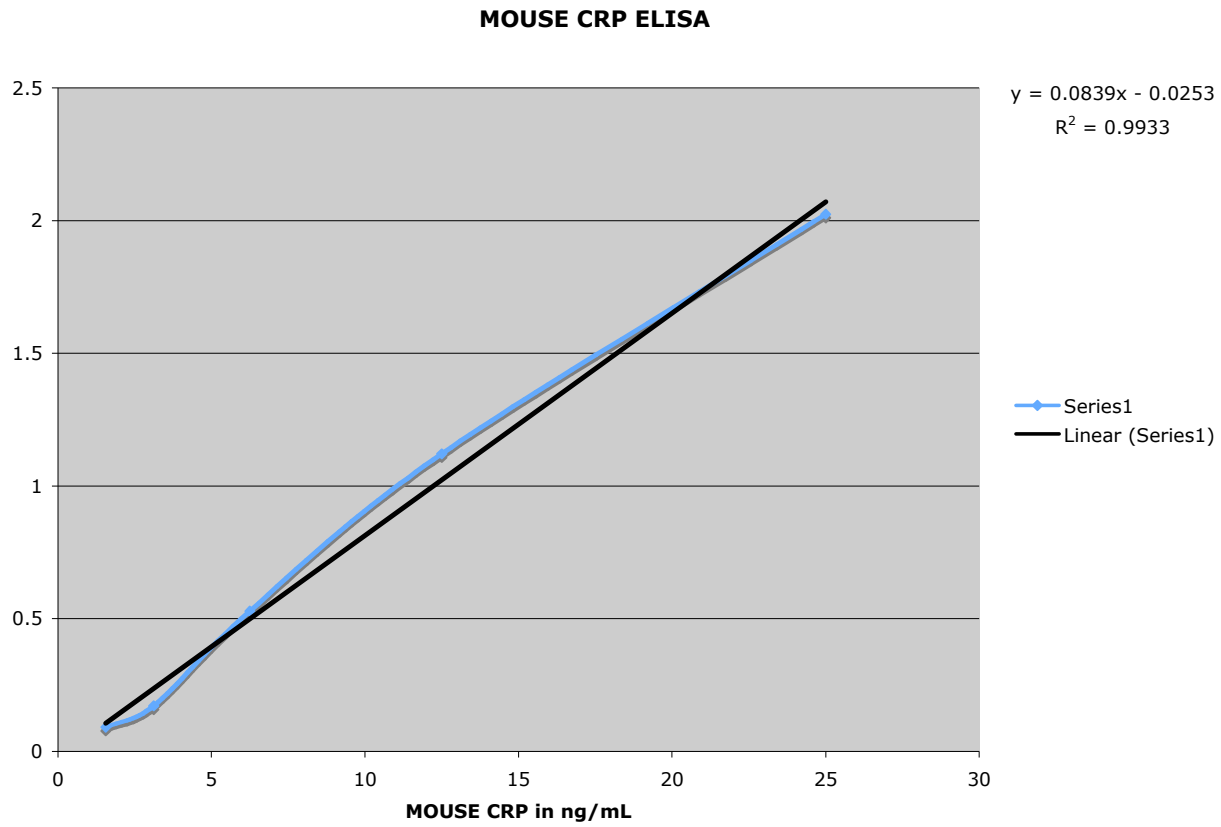
Standard #	Concentration	Volume Transferred	Diluent Volume	Total Volume	Final Volume (after transfer)
1	25.0 ng/mL	4 µL	400 µL	400 µL	200 µL
2	12.5 ng/mL	200 µL	200 µL	400 µL	200 µL
3	6.25 ng/mL	200 µL	200 µL	400 µL	200 µL
4	3.13 ng/mL	200 µL	200 µL	400 µL	200 µL
5	1.56 ng/mL	200 µL	200 µL	400 µL	400 µL

3. Sample preparation at 1:20: a) Dilute each serum sample 1:20 as follows: into a dilution vial, add 190 µL of wash buffer. To this, add 10 µL of serum.
4. Add 100 µL to each well and incubate at ambient temperature for 60 minutes. Record the location for later reference.

5. Wash plates 4 - 5 times with a gentle stream of wash buffer from a wash bottle or a plate washer. Tap plates on a stack of absorbent paper towels to remove residual buffer.
6. Dilute stock conjugate (100x) to the desired working dilution (1x) with the wash buffer, e.g. to 10 mL buffer, add 100 µL stock conjugate. This amount is sufficient for processing the whole plate. If only a part of the plate is being processed, the necessary aliquot has to be pipetted.
7. To each microwell, add 100 µL of conjugate.
8. Cover plate and incubate for 30 minutes at ambient temperature (20-25°C).
9. Wash plate as in step 5.
10. To each microwell, add 100 µL TMB/substrate solutions and allow reaction to proceed at ambient temperature for 5 - 10 minutes. A blue color indicates a positive reaction.
11. Stop reaction by adding 100 µL of Stop solution to each well. Reaction mixture turns from blue to yellow.
12. Read absorbance (OD) on a microplate reader equipped with a 450 nm filter. A differential filter of 630 nm can also be used. Construct standard curve and read off values for patient samples or unknowns. Multiply values by 20 to get actual serum concentration.

11. RESULTS

TYPICAL CALIBRATION CURVE



Mouse CRP (ng/mL)

Standard Curve used in the measurement of mouse CRP in serum

12. LIMITATIONS

Lipemic sera may interfere with specific antibody reaction.

13. QUALITY CONTROL

Routinely run at least two controls each giving values at the top or bottom regions of the standard curve respectively. An occasional prozone may be encountered in sera with high CRP values. In this situation, due to antigen excess, all the CRP available may not have reacted with the conjugate. Therefore, test at higher dilution, e.g. 1:40 and 1:80 to obtain more accurate results.

EXPECTED VALUES

A study performed on over 21 sera from healthy mice showed a range of 70 - 200 ng/mL CRP in serum. Similar results are expected on plasma. Data on urine and other fluids is not available.

14. PERFORMANCE CHARACTERISTICS

REPRODUCIBILITY

Inter-assay reproducibility (n=12, 2 plate lots)

ID	CRP (ng/mL)	CV (%)
1	25.0	6
2	12.5	7
3	6.25	6
4	3.13	5
5	1.56	8

Intra-assay reproducibility (n=12)

ID	CRP (ng/mL)	CV (%)
1	25.0	3
2	12.5	3
3	6.25	6
4	3.13	4
5	1.56	6

SENSITIVITY

The mouse CRP assay is designed to detect elevated levels of CRP. The following data was produced to generate data on the sensitivity of the assay and maybe useful in research applications where sensitivity parameters need to be defined.

Assay Sensitivity n=11

Sample	Mean [OD]	Standard Variation	Detection Limit ([ng/ml)
1	0.087	0.008	2.5

CROSS REACTIVITY

Rat CRP	100%

NOTES

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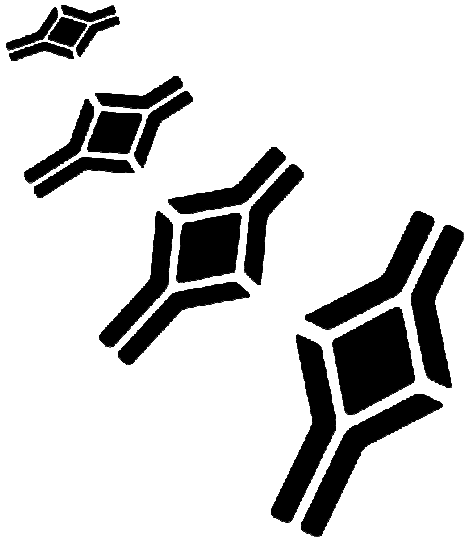

HEADQUARTERS: **BioVendor Laboratorní medicína, a.s.**	**CTPark Modrice Evropska 873**	**664 42 Modrice CZECH REPUBLIC**	**Phone: +420-549-124-185 Fax: +420-549-211-460**	**E-mail:info@biovendor.com Web:www.biovendor.com**
EUROPEAN UNION: BioVendor GmbH	Im Neuenheimer Feld 583	D-69120 Heidelberg GERMANY	Phone: +49-6221-433-9100 Fax: +49-6221-433-9111	E-mail: infoEU@biovendor.com
USA, CANADA AND MEXICO: BioVendor LLC	1463 Sand Hill Road Suite 227	Candler, NC 28715 USA	Phone: +1-828-670-7807 +1-800-404-7807 Fax: +1-828-670-7809	E-mail: infoUSA@biovendor.com
CHINA - Hong Kong Office: BioVendor Laboratories Ltd	Room 4008 Hong Kong Plaza, No.188	Connaught Road West Hong Kong, CHINA	Phone: +852-2803-0523 Fax: +852-2803-0525	E-mail: infoHK@biovendor.com
CHINA – Mainland Office: BioVendor Laboratories Ltd	Room 2405 YiYa Tower TianYu Garden, No.150	Lihe Zhong Road Guang Zhou, CHINA	Phone: +86-20-8706-3029 Fax: +86-20-8706-3016	E-mail: infoCN@biovendor.com