BioVendor Group

ENG

Instructions for Use:
RAT CRP ELISA

Catalogue number:
REH002R

For research use only!

BioVendor R&D

BioVendor – Laboratorní medicína a.s.
Karásek 1767/1, 621 00 Brno, Czech Republic
+420 549 124 185
info@biovendor.com
sales@biovendor.com
www.biovendor.com

CONTENT

HISTORY OF CHANGES

Previous version	Current version
ENG.001.A	ENG.002.A
Change of component designation.	
ENG.002.A	ENG.002.B
Chapter 5, point 4: Add 150 µl CRP standard from the vial Standard Protein, into a tube with 350 µl 1X Assay Diluent to prepare a 60 ng/ml standard solution.	Chapter 5, point 4: Add 150 µl CRP standard from the standard vial, into a tube with 350 µl 1X Assay Diluent to prepare a 60 ng/ml standard solution

1. INTRODUCTION

The BioVendor Rat CRP (C Reactive Protein) ELISA kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of Rat CRP in serum, plasma, cell culture supernatants. This assay employs an antibody specific for Rat CRP coated on a 96-well plate. Standards and samples are pipetted into the wells and CRP present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated antiRat CRP antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of CRP bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

2. STORAGE, EXPIRATION

The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C. For prepared reagent storage, see table below.

3. REAGENT SUPPLIED

Component	Size / Description	Storage / Stability After Preparation
CRP Microplate	96 wells (12 strips x 8 wells) coated with anti-Rat CRP	1 month at 4 °C
Standard Protein	2 vials of Rat CRP. 1 vial is enough to run each standard in duplicate	1 week at -80 °C
Detection Antibody CRP	2 vials of biotinylated anti-Rat CRP. Each vial is enough to assay half the microplate	5 days at 4 °C
Wash Buffer	25 ml of 20X concentrated solution	1 month at 4 °C
HRP-Streptavidin	200 µl 500X concentrated HRP-conjugated streptavidin	Do not store and reuse
TMB One-Step Substrate Reagent	12 ml of 3,3,5,5´-tetramethylbenzidine (TMB) in buffer solution	N/A
Stop Solution	8 ml of 0.2 M sulfuric acid	N/A
5X Assay Diluent B	2 bottles of 15ml 5X concentrated buffer	1 month at 4 °C

Note: Return unused wells to the pouch containing desiccant pack, reseal along entire edge.

4. MATERIAL REQUIRED BUT NOT SUPPLIED

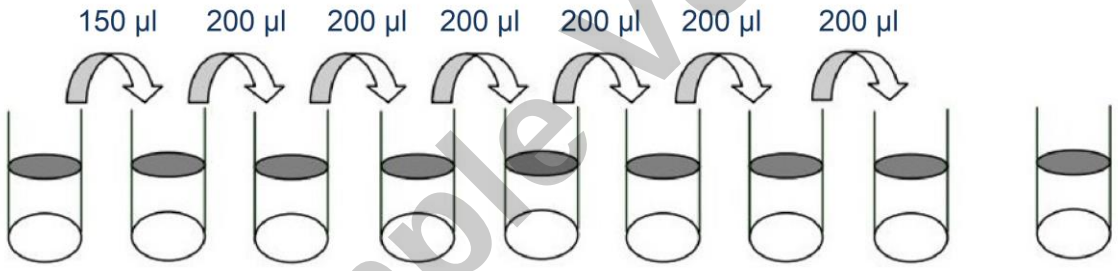
1. Microplate reader capable of measuring absorbance at 450 nm.
2. Precision pipettes to deliver 2 µl to 1 ml volumes.
3. Adjustable 1-25 ml pipettes for reagent preparation.
4. 100 ml and 1 liter graduated cylinders.
5. Absorbent paper.
6. Distilled or deionized water.
7. Log-log graph paper or computer and software for ELISA data analysis.
8. Tubes to prepare standard or sample dilutions.

5. PREPARATION OF REAGENTS

1. Bring all reagents and samples to room temperature (18 - 25ºC) before use.
2. 5X Assay Diluent B should be diluted 5-fold with deionized or distilled water before use.
3. Sample dilution: 1X Assay Diluent B should be used for dilution of serum, plasma, and cell culture supernatant samples. The suggested dilution for normal serum/plasma is 20,000 - 200,000 fold. For example, add 1 µl of serum/plasma into a tube with 99 µl 1X Assay Diluent to prepare a 100-fold diluted sample. Mix thoroughly and then pipette 1 µl of prepared 100-fold diluted sample into a tube with 499 µl 1X Assay Diluent to prepare a final 50,000 fold diluted sample.

   Note: Levels of CRP may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.

4. Preparation of standard: Briefly spin a vial of standard protein. Add 400 µl 1X Assay Diluent B into Standard Protein vial to prepare a 200 ng/ml standard solution. Dissolve the powder thoroughly by a gentle mix. Add 150 µl CRP standard from the standard vial, into a tube with 350 µl 1X Assay Diluent to prepare a 60 ng/ml standard solution. Pipette 300 µl 1X Assay Diluent B into each tube. Use the 60 ng/ml standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. 1X Assay Diluent B serves as the zero standard (0 pg/ml).



		Std1	Std2	Std3	Std4	Std5	Std6	Std7		Zero Standard
Diluent volume	Standard +400 µl	350 µl	300 µl	300 µl	300 µl	300 µl	300 µl	300 µl		300 µl
Conc.	200 ng/ml	60 ng/ml	24 ng/ml	9.6 ng/ml	3.84 ng/ml	1.536 ng/ml	0.614 ng/ml	0.246 ng/ml		0 ng/ml

5. If the Wash Buffer (20X) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1X Wash Buffer.
6. Briefly spin the Detection Antibody vial before use. Add 100 µl of 1X Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4ºC for 5 days). The detection antibody concentrate should be diluted 80-fold with 1X Assay Diluent B and used in step 5 of Assay Procedure.
7. Briefly spin the HRP-Streptavidin concentrate vial and pipette up and down to mix gently before use, as precipitates may form during storage. HRP-Streptavidin concentrate should be diluted 500-fold with 1X Assay Diluent B.

   For example: Briefly spin the HRP-Streptavidin vial and pipette up and down to mix gently. Add 20 µl of HRP-Streptavidin concentrate into a tube with 10 ml 1X Assay Diluent B to prepare a 500-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

6. ASSAY PROCEDURE

1. Bring all reagents and samples to room temperature (18 - 25ºC) before use. It is recommended that all standards and samples be run at least in duplicate.
2. Label removable 8-well strips as appropriate for your experiment.
3. Add 100 µl of each standard (see Reagent Preparation step 4) and sample into appropriate wells. Cover wells and incubate for 2.5 hours at room temperature with gentle shaking.
4. Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (300 µl) using a multi-channel Pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100 µl of 1X prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.
6. Discard the solution. Repeat the wash as in step 4.
7. Add 100 µl of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking.
8. Discard the solution. Repeat the wash as in step 4.
9. Add 100 µl of TMB One-Step Substrate Reagent to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
10. Add 50 µl of Stop Solution to each well. Read at 450 nm immediately.

7. ASSAY PROCEDURE - SUMMARY

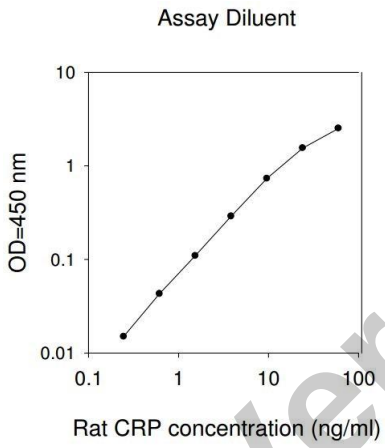
1. Prepare all reagents, samples and standards as instructed.
2. Add 100 µl standard or sample to each well. Incubate 2.5 hours at room temperature.
3. Add 100 µl prepared biotin antibody to each well. Incubate 1 hour at room temperature.
4. Add 100 µl prepared Streptavidin solution. Incubate 45 minutes at room temperature.
5. Add 100 µl TMB One-Step Substrate Reagent to each well. Incubate 30 minutes at room temperature.
6. Add 50 µl Stop Solution to each well. Read at 450 nm immediately.

8. CALCULATIONS

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

8.1 Typical Data

These standard curves are for demonstration only. A standard curve must be run with each assay.



8.2 Sensitivity

The minimum detectable dose of Rat CRP was determined to be 200 pg/ml.

Minimum detectable dose is defined as the analyte concentration resulting in an absorbance that is 2 standard deviations higher than that of the blank (diluent buffer).

8.3 Spiking & Recovery

Recovery was determined by spiking various levels of Rat CRP into the sample types listed below. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Serum	98.54	86-108
Plasma	121.5	109-142
Cell culture media	112.2	104-120

8.4 Linearity

Sample Type		Serum	Plasma	Cell Culture Media
1:2	Average % of Expected	82.16	101.7	101.1
	Range (%)	70-94	93-120	93-109
1:4	Average % of Expected	86.50	75.42	83.48
	Range (%)	80-92	67-83	68-90

8.5 Reproducibility

Intra-Assay CV%: <10%

Inter-Assay CV%: <12%

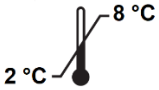
8.6 Specifity

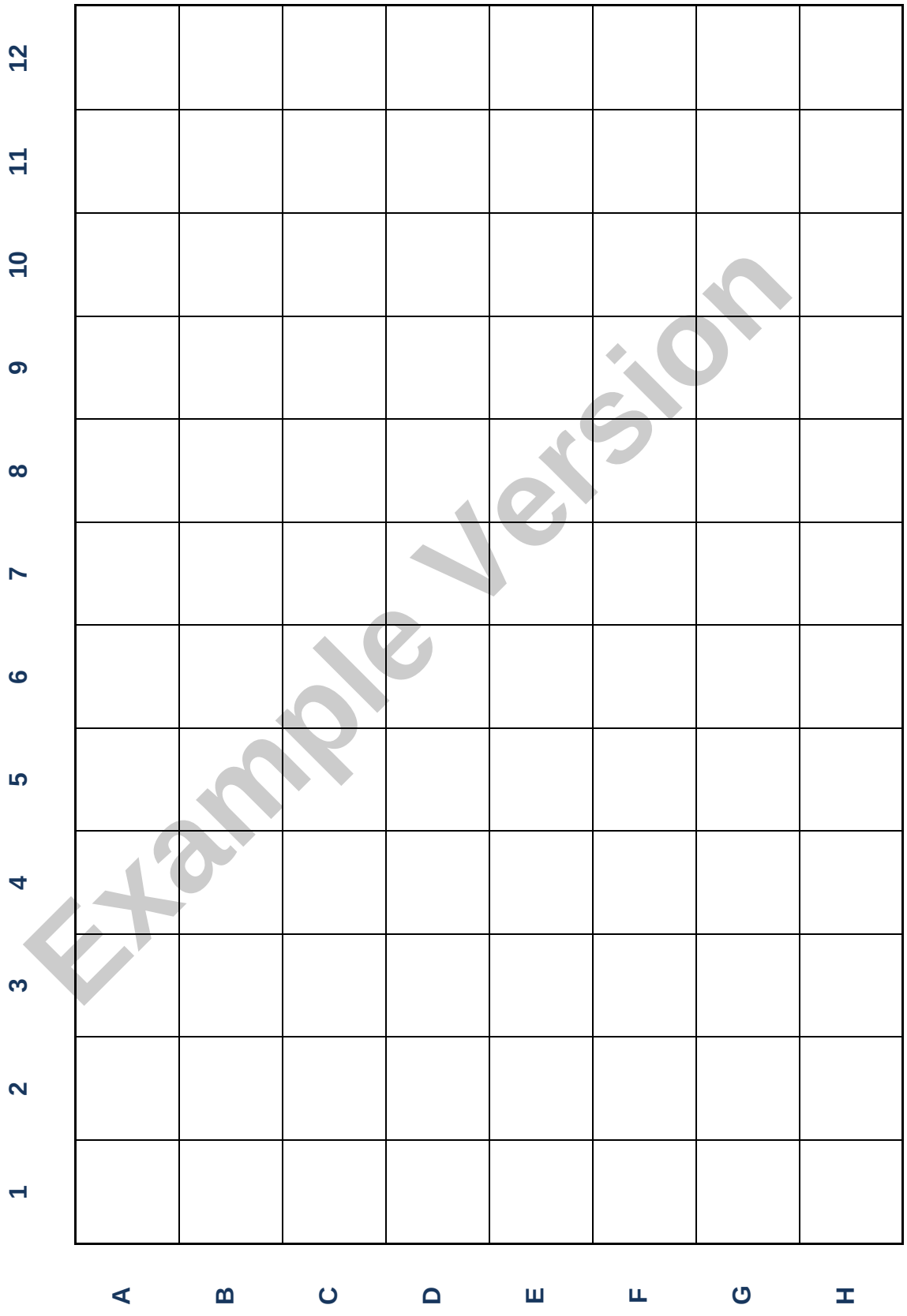
This ELISA kit shows no cross-reactivity with the following cytokines tested: rat CINC-2, CINC-3, CNTF, Fractalkine, IL-1 alpha, IL-1 beta, IL-4, IL-6, IL-10, GM-CSF, IFN-gamma, Leptin, Lix, MCP-1, MIP-3 alpha, beta- NGF, TIMP-1, TNF-alpha

9. TROUBLESHOOTING

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standard dilution	Briefly centrifuge the standard protein and dissolve the powder thoroughly by gently mixing
Low signal	Improper preparation of standard and/or biotinylated antibody	Briefly spin down before opening. Dissolve the powder thoroughly.
	Too brief incubation times	Ensure sufficient incubation time. Assay procedure step 3 may be done overnight at 4 °C with gentle shaking (note: may increase overall signals including background)
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Inaccurate pipetting	Check pipettes
	Air bubbles in wells	Remove bubbles in wells
High background	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, ensure that all ports are unobstructed
	Contaminated wash buffer	Make fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store your standard at <-70 °C after reconstitution, others at 4 °C. Keep substrate solution protected from light
	Stop solution	Add stop solution to each well before reading plate

10. EXPLANATION OF SYMBOLS

	Catalogue number
	Batch code
	Caution
	Use by date
	Temperature limit
	Manufacturer
	Read electronic instructions for use - eIFU
	The content is sufficient for 96 tests
	Biological risks



BioVendor – Laboratorní medicína a.s.
Karásek 1767/1, 621 00 Brno, Czech Republic
+420 549 124 185
info@biovendor.com
sales@biovendor.com
www.biovendor.com