BioVendor Group

ENG

Instructions for Use:

HUMAN MONOMERIC CRP (mCRP) ELISA

Catalogue number:

RBL010R

For research use only!

BioVendor R&D

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CONTENT

HISTORY OF CHANGES

Previous version	Current version
	ENG.001.A
New edition	

1. INTENDED USE

Enzyme Immunoassay for the quantitative determination of monomeric CRP (mCRP) in human serum and plasma.

2. STORAGE, EXPIRATION

– The kit must be stored at 2 – 8°C.
– The opened components can be stored for one week at 2 – 8°C.

3. INTRODUCTION

C-reactive protein (CRP) is a multipotent protein that undergoes conformational changes between circulating native pentameric CRP (pCRP), pentameric symmetrical forms (pCRP*) and monomeric CRP (mCRP) forms. mCRP exhibits strong pro-inflammatory activity and activates platelets, leukocytes, and endothelial cells. Abundant deposition of mCRP in inflamed tissues plays a role in several disease conditions, such as ischemia/reperfusion injury, Alzheimer's disease, and cardiovascular disease.
Conversion of pCRP to mCRP induces inflammatory signalling, monoacyl phosphatidylcholine generated by PLA2, or by oxidation lipid acyl chains, promotes binding and dissociation of pCRP to mCRP. mCRP gains functionally active neoepitopes that carry out highly pro-inflammatory and pro-thrombotic features, thus mCRP can bind cholesterol and enter plasmatic membrane and activate pro-inflammatory responses.
Deposition of mCRP, which has a much lower aqueous solubility than pCRP, has been shown in the brain in infarcted areas of Alzheimer diseases patients and in regions with amyloid burden, in atherosclerotic plaques in vascular disease and in other foci of inflammatory tissue injuries.

4. TEST PRINCIPLE

The microtiter plate is coated with the antibody specifically binding the mCRP. The human serum and plasma are incubated in the plate with the capture antibody.
The specimen is washed out and the specifically bound protein is incubated with biotin-labelled detection antibody. Following another washing step, Streptavidin-HRP conjugate is added into the well.
Unbound reagent is then washed out. Horseradish peroxidase (HRP) bound in the complex reacts with the chromogenic substrate (TMB) creating the blue colour. The reaction is stopped by addition of STOP solution (H_2SO_4).
The absorbance values are measured at 450 nm (optionally 450/630 nm) and are proportional to the concentration of mCRP in the specimen. The concentration of mCRP in unknown samples is determined from the calibration curve which is created by plotting the absorbance values against the standard concentration values.

5. PRECAUTIONS

- For research use only
- For professional laboratory use
- The reagents with different lot numbers should not be mixed
- To prevent cross sample contamination, use disposable labware and pipette tips
- To protect laboratory stuff, wear protective gloves and protective clothing
- The substrate solution should remain colourless, keep it protected from light
- The test should be performed at standard laboratory conditions (temperature 25°C ± 2°C).

6. REAGENT SUPPLIED

Item	Qty.
Antibody Coated Microtiter Plate	96 wells
Biotin-labelled Antibody	13 mL
Streptavidin-HRP Conjugate	13 mL
Master Standard	1 vial
Quality Control A (human serum, lyophilized)	1 vial
Quality Control B (human serum, lyophilized)	1 vial
Dilution Buffer	2x13 mL
Wash Buffer 15x conc.	50 mL
Substrate Solution	13 mL
STOP Solution	13 mL

7. MATERIAL REQUIRED BUT NOT SUPPLIED

- Glassware and test tubes
- Microtiter plate washer
- Precision pipettes (various volumes) with tips
- Orbital shaker
- Microtiter plate reader capable of measuring absorbance at 450 nm or 450/630 nm with software for data generation

8. PREPARATION OF REAGENTS

Use new pipette tip for pipetting different reagents and samples to prevent cross-contamination. All reagents and samples should be allowed to reach the temperature 25°C ± 2°C.

8.1 Preparation of Standards

Reconstitute lyophilized Human mCRP Master Standard in Dilution Buffer, for the volume information see the Certificate of Analysis. Let it rehydrate for 15 min. The concentration of human mCRP Master Standard is 80 ng/mL.
Prepare set of Standard solution as follows:
Use the Master Standard to produce a dilution series (as below). Mix each tube thoroughly before the next transfer. The Dilution Buffer serves as Blank.

	Volume of Standard	Dilution Buffer	Concentration
Std1	Standard 80 ng/mL (lyophilized)	See CofA	80 ng/mL
Std2	300 µL of Std1	300 µL	40 ng/mL
Std3	300 µL of Std2	300 µL	20 ng/mL
Std4	300 µL of Std3	300 µL	10 ng/mL
Std5	300 µL of Std4	300 µL	5 ng/mL
Std6	300 µL of Std5	300 µL	2.5 ng/mL
Std7	300 µL of Std6	300 µL	1.25 ng/mL
Blank	-	300 µL	0 ng/mL

8.2 Preparation of Quality Control A and B

Reconstitute the lyophilized human serum Quality Controls in deionized/distilled water, for the volume information see the Certificate of Analysis. Let the QCs rehydrate for 15 min and dilute them 1:2 prior to use, see Preparation of samples.

8.3 Preparation of Wash Buffer 1x

Prepare a working solution of Wash Buffer by adding 50 mL of Wash Buffer 15x conc. to 700 mL of deionized/ distilled water (dH_2O). Mix well. Store at 4°C for two weeks or at -20°C for long term storage.

9. PREPARATION OF SAMPLES

Human serum and plasma may be used with this assay. For long-term storage the samples should be frozen at minimum -70ºC. Lipemic or haemolytic samples may cause false results.
Recommended dilution of samples is 1:2, i.e., for singlets 80 µL of sample + 80 µL of Dilution Buffer, for duplicates 150 µL of samples + 150 µL of Dilution Buffer, respectively.

10. ASSAY PROCEDURE

1. Prepare the reagents as described in the previous chapter.
2. Pipette 100 µL of set of Standards, Quality Controls, diluted Samples and Dilution Buffer = Blank into each well. Incubate for **OVERNIGHT** at 4°C ±2°C, NO shaking.
3. Wash the wells **3-times** with 1x Wash Buffer (350 µL/well). When finished, tap the plate against the paper towel to remove the liquid completely.
4. Pipette 100 µL of Biotin-labelled Antibody into each well. Incubate for **1 hour** at 25°C ±2°C, shaking at 350 rpm.
5. Wash the wells **5-times** with 1x Wash Buffer (350 µL/well). When finished, tap the plate against the paper towel to remove the liquid completely.
6. Pipette 100 µL of Streptavidin-HRP into each well. Incubate for **30 min** at 25°C ±2°C, shaking at 300 rpm.
7. Wash the wells **5-times** with 1x Wash Buffer (350 µL/well). When finished, tap the plate against the paper towel to remove the liquid completely.
8. Pipette 100 µL Substrate solution, incubate for **30 min** at 25°C ±2°C. Avoid exposure to the light during this step.
9. Pipette 100 µL of STOP solution.
10. Read the signal at 450 or 450/630 nm within 15 min.

11. PERFORMANCE CHARACTERISTICS

Samples used in the tests were diluted 1:2 as recommended and assayed. The results are multiplied by the dilution factor.

11.1 Sensitivity

The limit of detection, defined as a concentration of human MCRP giving absorbance higher than absorbance of blank + 3 standard deviations, is better than 0.63 ng/mL of sample.

11.2 Precision

11.2.1 Intra-assay

Sample	Mean (ng/mL)	SD	CV (%)
1	2.1	0.1	4.3
2	2.4	0.2	8.1

11.2.2 Inter-assay (Run – to – run)

Sample	Mean (ng/mL)	SD	CV (%)
1	46.1	6.7	14.5
2	8.8	0.4	5.0

11.3 Accuracy

11.3.1 Dilution linearity

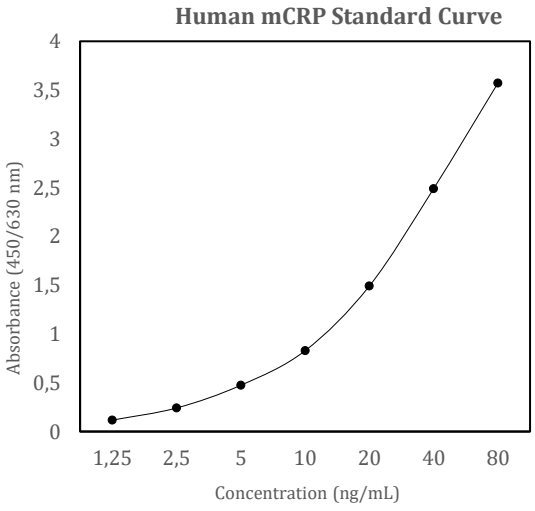
Sample	Dilution	Measured concentration (ng/mL)	Expected concentration (ng/mL)	Yield (%)
1		39.4	-	-
	2x	18.7	196.6	95
	4x	9.5	98.5	96
	8x	4.7	49.2	96
2		18.6	-	-
	2x	96.5	92.9	104
	4x	46.6	46.4	100
	8x	22.6	23.2	97

11.3.2 Spiking Recovery

Sample	Spike (Ng/mL)	Measured concentration (ng/mL)	Expected concentration (ng/mL)	Yield (%)
1	-	3.27	-	-
	20	20.0	23.3	86
	10	11.6	13.3	88
	5	7.2	8.3	87

12. CALCULATION

The standard curve needs to be measured in every test. Most of the microplate reader can automatically calculate the analyte concentration using 4-parameter algorithm or alternative functions to fit the standard points properly. The concentrations need to be multiplied by the dilution factor, either automatically by reader or manually.



13. POPULATION AND CLINICAL DATA

Typical mCRP values in human serum were obtained with BioLab Assays Human Monomeric CRP (mCRP) ELISA kit.

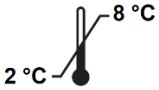
Individuals with hsCRP 1-5mg/L	1.2-4.8 ng/mL (median 2.6 ng/mL)
Individuals with pancreatic cancer	18.3 – 73.9 ng/mL (median 36.5 ng/mL)
Individuals with bacterial infection, CRP over 50mg/L	24.1 – 98.3 ng/mL (median 47.7 ng/mL)

According to the health status, mCRP next to mCRP/pCRP or mCRP/hsCRP ratio should be taken into consideration to evaluate a risk value.

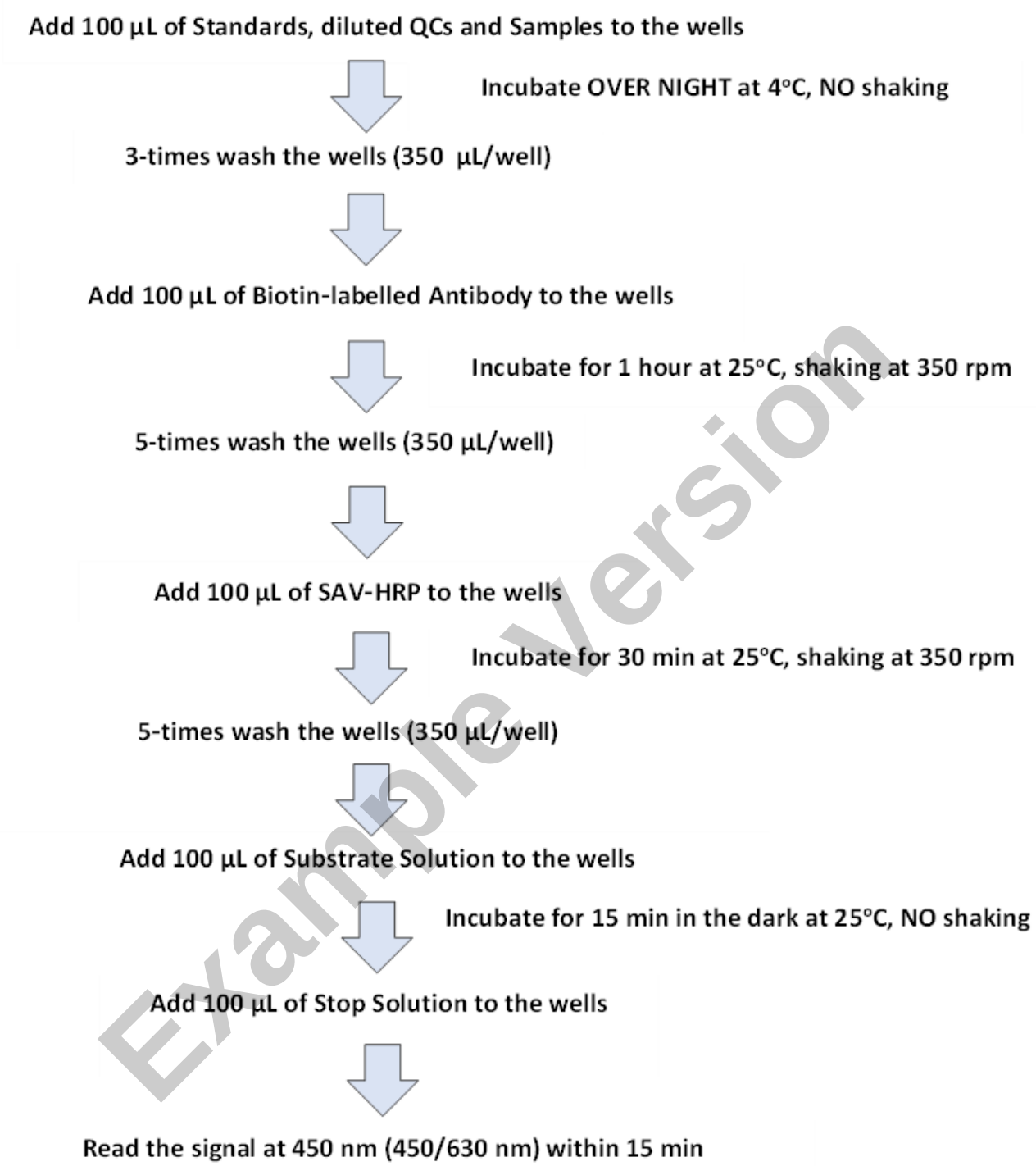
14. REFERENCES

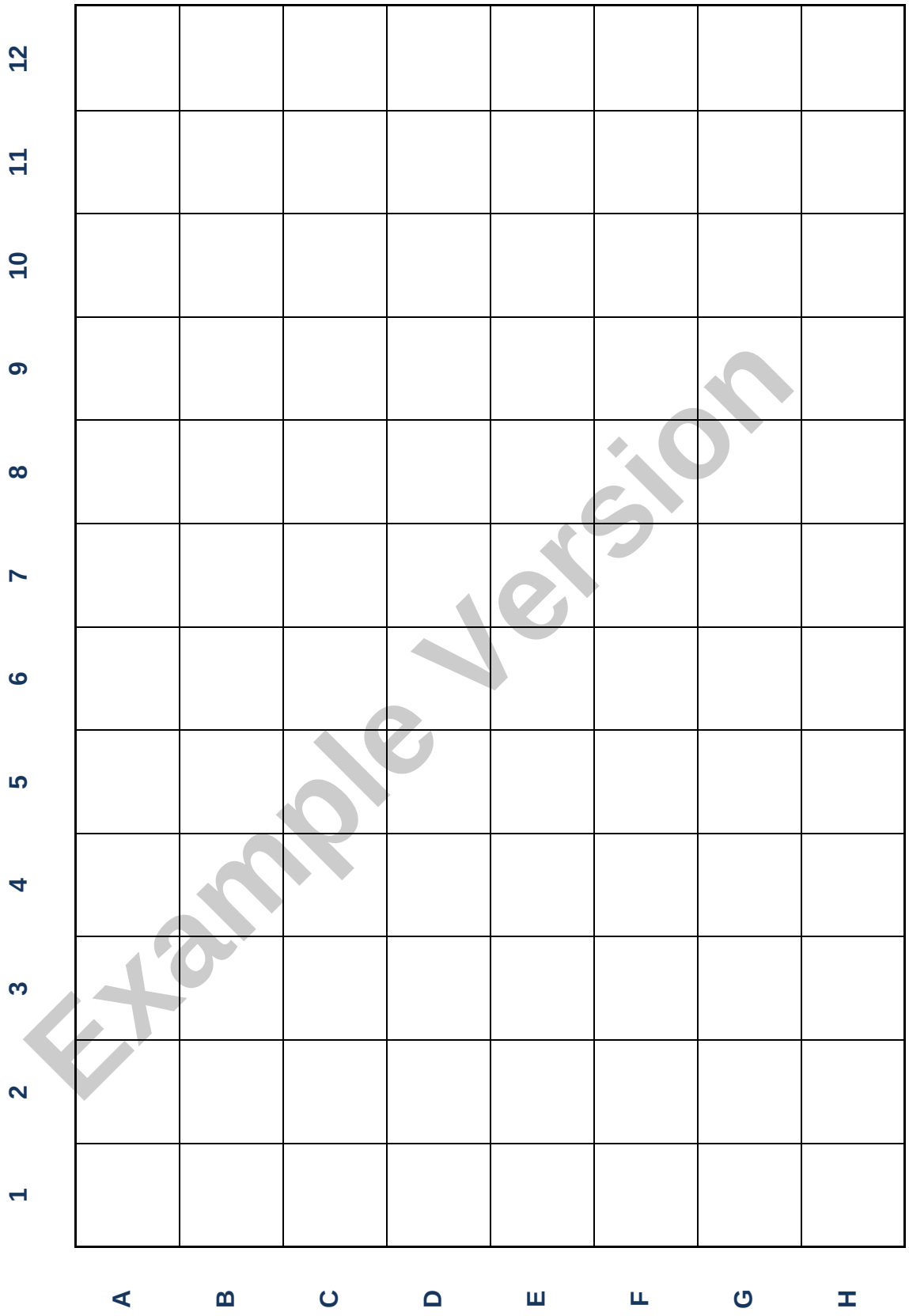
[1] Garcia-Lara E, Aguirre S, Clotet N, Sawkulycz X, Bartra C, Almenara-Fuentes L, et al. Antibody Protection Against Long-Term Memory Loss Induced by Monomeric C-Reactive Protein in a Kouse Model of Dementia. Biomedicines 9(7):828 (2021)

15. 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

16. ASSAY PROCEDURE - SUMMARY





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