Manufactured for:
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High Sensitivity C-Reactive Protein (hs-CRP) ELISA

REF IB59126	RUO
Effective Date: August 15, 2023	Version: RUO-7.0

1. INTENDED PURPOSE & USE

For the quantitative measurement of C-Reactive Protein (CRP) in human serum by an ELISA (Enzyme-Linked Immunosorbent Assay).

For Research Use Only. Not for use in diagnostic procedures.

2. LIMITATIONS RELATED TO INTENDED PURPOSE & USE

1. This kit is intended for research use only and is not to be used for any diagnostic procedures.

3. PRINCIPLE OF THE TEST

The hs-CRP ELISA is a two-step capture or 'sandwich' type immunoassay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for CRP is immobilized onto the microplate and another monoclonal antibody specific for a different epitope of CRP is conjugated to horse radish peroxidase (HRP conjugate). In the first incubation step, CRP present in the specimen samples, calibrators and controls is bound by the antibody immobilized onto the microplate. Excess and unbound materials are removed by a washing step.
In the second incubation step, HRP conjugate antibody (HRP conjugate) is added, which binds specifically to any immobilized CRP, thus forming a sandwich complex. Unbound HRP conjugate is removed by a washing step. Next, the TMB substrate (enzyme substrate) is added which reacts with HRP to form a blue coloured product that is directly proportional to the amount of CRP present. The enzymatic reaction is terminated by the addition of the stopping solution, converting the colour from blue to yellow. The absorbance is measured on a microplate reader at 450 nm. A set of calibrators is used to plot a calibrator curve from which the amount of CRP in specimen samples and controls can be directly read.

4. PROCEDURAL CAUTIONS AND WARNINGS

1. This kit is for use by trained laboratory personnel (professional use only). For laboratory *in vitro* use only.
2. Practice good laboratory practices when handling kit reagents and specimens. This includes:
   - Do not pipette by mouth.
   - Do not smoke, drink, or eat in areas where specimens or kit reagents are handled.
   - Wear protective clothing and disposable gloves.
   - Wash hands thoroughly after performing the test.
   - Avoid contact with eyes; use safety glasses; in case of contact with eyes, flush eyes with water immediately and contact a doctor.
3. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
4. Do not use the kit beyond the expiry date stated on the label.
5. If the kit reagents are visibly damaged, do not use the test kit.
6. Do not use kit components from different kit lots within a test and do not use any component beyond the expiration date printed on the label.
7. All kit reagents and specimens must be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of specimens.
8. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
9. Immediately after use, each individual component of the kit must be returned to the recommended storage temperature stated on the label.
10. A calibrator curve must be established for every run.
11. It is recommended to all customers to prepare their own control materials or serum pools which should be included in every run at a high and low level for assessing the reliability of results.
12. The controls (included in kit) must be included in every run and their results must fall within the ranges stated in the quality control certificate; a failed control result might indicate improper procedural techniques or pipetting, incomplete washing, or improper reagent storage.
13. When dispensing the substrate and stopping solutions, do not use pipettes in which these liquids will come into contact with any metal parts.
14. The TMB Substrate is sensitive to light and should remain colourless if properly stored. Instability or contamination may be indicated by the development of a blue colour, in which case it should not be used.
15. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
16. Samples or controls containing azide or thimerosal are not compatible with this kit, they may lead to false results.
17. Samples values above the measuring range of the kit may be reported as >10,000 ng/mL. If further dilution and retesting is required, only calibrator A may be used to dilute serum samples. The use of any other reagent may lead to false results.
18. Avoid microbial contamination of reagents.
19. To prevent the contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, calibrator, and control.
20. To prevent the contamination of reagents, do not pour reagents back into the original containers.
21. Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.
22. Consumables used with the kit that are potentially biohazardous (e.g., pipette tips, bottles or containers containing human materials) must be handled according to biosafety practices to minimize the risk of infection and disposed of according to local and/or national regulations relating to biohazardous waste.
23. This kit contains 1 M sulfuric acid in the stopping solution component. Do not combine acid with waste material containing sodium azide or sodium hypochlorite.
24. The use of safety glasses, and disposable plastic, is strongly recommended when manipulating biohazardous or bio-contaminated solutions.
25. Proper calibration of the equipment used with the test, such as the pipettes and absorbance microplate reader, is required.
26. If a microplate shaker is required for the assay procedure, the type and speed of shaker required is stated in the REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED section. Both the type and speed of shaker used can influence the optical densities and test results. If a different type of shaker and/or speed is used, the user is responsible for validating the performance of the kit.
27. Do not reuse the microplate wells, they are for SINGLE USE only.
28. To avoid condensation within the microplate wells in humid environments, do not open the pouch containing the microplate until it has reached room temperature.
29. When reading the microplate, the presence of bubbles in the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.

5. SAFETY CAUTIONS AND WARNINGS

5.1 BIOHAZARDS

The reagents should be considered a potential biohazard and handled with the same precautions applied to blood specimens. All human specimens should be considered a potential biohazard and handled as if capable of transmitting infections and in accordance with good laboratory practices.

The calibrators and controls provided with the kit contain material(s) of human origin that have been tested and found to be negative for the presence for HIV-1, HIV-2, Hepatitis B and Hepatitis C. However, no test method can offer complete assurance that any viable pathogens are absent. Therefore, these components should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen, following good laboratory practices.

5.2 CHEMICAL HAZARDS

Avoid direct contact with any of the kit reagents. Specifically avoid contact with the TMB Substrate (contains tetramethylbenzidine) and Stopping Solution (contains sulfuric acid). If contacted with any of these reagents, wash with plenty of water and refer to SDS for additional information.

6. SPECIMEN COLLECTION, STORAGE AND PRE-TREATMENT

6.1 Specimen Collection & Storage

Approximately 0.1 mL of serum is required per duplicate determination. Collect 4–5 mL of venous blood into an appropriately labelled tube and allow it to clot at room temperature. Centrifuge at room temperature and carefully transfer the serum into a new labelled storage tube or container. Serum samples may be stored at 2-8°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date.

Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

6.2 Specimen Pre-Treatment & Storage

All serum specimens must be diluted 1:20 in calibrator A before being used in the test. Follow the specimen pre-treatment procedure as stated below for each specimen that is to be tested:

1. Pipette 190 µL of calibrator A into a new polypropylene, HDPE or disposable glass tube.
2. Pipette 10 µL of the serum specimen into the tube from step 1 that contains 190 µL of calibrator A.
3. Close the tube and label with specimen identification information.
4. Mix the contents of the tube by vortexing.

Do not pre-treat the calibrators and kit controls; they are provided in a ready to use format.

Note: Different volumes of Calibrator A and serum specimen may be used provided that the required 1:20 ratio is maintained (1 part serum specimen to 19 parts Calibrator A).

Diluted samples (1:20 dilution used in the test) should be used within 2 hours following preparation. Do not store diluted specimens beyond this time limit.

7. REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Calibrated single-channel pipette to dispense 10 µL, 20 µL and 190 µL.
2. Calibrated multi-channel pipettes to dispense 50 µL, 100 µL and 200 µL.
3. Calibrated multi-channel pipettes to dispense 300 µL (if washing manually).
4. Automatic microplate washer (recommended).
5. Microplate shaker:
   a. Orbital shaker (3 mm diameter) set to 600 rpm or
   b. Reciprocating shaker (1.5" stroke length) set to 180 oscillations/minute.
6. Disposable pipette tips.
7. Distilled or deionized water.
8. Calibrated absorbance microplate reader with a 450 nm filter and an upper OD limit of 3.0 or greater.
9. Polypropylene/HDPE or disposable glass tubes for sample pre-treatment.
10. Vortex mixer.

8. REAGENTS PROVIDED

1. MPL — Microplate

Contents:	One anti-CRP monoclonal antibody-coated 96-well (12x8) microplate in a resealable pouch with desiccant.
Format:	Ready to Use
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks.

2. HRP CONJ CONC — HRP Conjugate Concentrate

Contents:	One bottle containing anti-CRP monoclonal antibody-Horse Radish Peroxidase (HRP) conjugate in a protein-based buffer with a non-mercury preservative.
Format:	Concentrated; Requires Preparation
Volume:	0.3 mL/bottle
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks.
Preparation of HRP Conjugate Working Solution:	**X81 — Dilute 1:81 Before Use** Dilute 1:81 in assay buffer before use (e.g., 25 µL of HRP conjugate concentrate in 2 mL of assay buffer). If the whole plate is to be used dilute 150 µL of HRP conjugate concentrate in 12 mL of assay buffer. Discard any that is left over.

3. CAL A – F — Calibrator A – F

Contents:	Six bottles of calibrator containing specified CRP concentrations. Protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of CRP. Calibrated against World Health Organization (WHO) IS 85/506. Listed below are approximate concentrations, please refer to vial labels for exact concentrations. Concentrations: 0, 100, 400, 1000, 4000, 10,000 ng/mL.
Format:	Ready to Use
Volume:	Calibrator A: 16 mL/bottle Calibrator B-F: 0.5 mL/bottle
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks.

4. CONTROL 1 – 2 — Control 1 – 2

Contents:	Two bottles of control containing different CRP concentrations. Protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of CRP. Refer to the QC certificate for the target values and acceptable ranges.
Format:	Ready to Use
Volume:	0.5 mL/bottle
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks.

5. ASY | BUFF — Assay Buffer

Contents:	One bottle containing a protein-based buffer with a non-mercury preservative.
Format:	Ready to Use
Volume:	40 mL/bottle
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks.

6. TMB | SUB — TMB Substrate

Contents:	One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.
Format:	Ready to Use
Volume:	16 mL/bottle
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks.

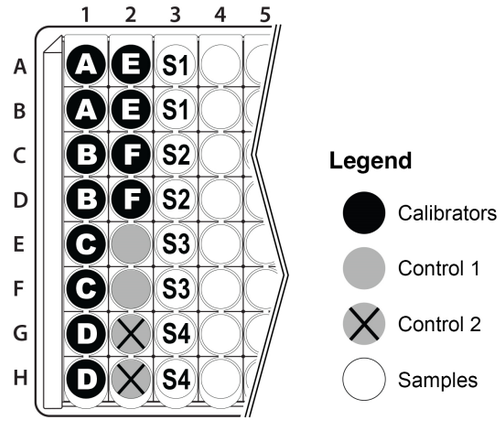
7. STOP — Stopping Solution

Contents:	One bottle containing 1M sulfuric acid.
Format:	Ready to Use
Volume:	6 mL/bottle
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks.
Safety:	Refer to product SDS. **Warning**

8. WASH | BUFF | CONC — Wash Buffer Concentrate

Contents:	One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
Format:	Concentrated; Requires Preparation
Volume:	50 mL/bottle
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks. Following Preparation: The wash buffer working solution is stable for 2 weeks following preparation, assuming Good Laboratory Practices are adhered to. To prevent microbial growth, prepare the wash buffer working solution in a clean container and store under refrigerated conditions (2-8°C) when not in use.
Preparation of Wash Buffer Working Solution:	**X10** **Dilute 1:10 Before Use** Dilute 1:10 in distilled or deionized water before use. If the whole microplate is to be used dilute 50 mL of the wash buffer concentrate in 450 mL of distilled or deionized water.

9. RECOMMENDED ASSAY LAYOUT



10. ASSAY PROCEDURE

Specimen Pre-Treatment:

All specimens that will be tested must be pre-treated before being tested (see section *6.2. Specimen Pre-Treatment & Storage*). Do not pre-treat the calibrators and kit controls as they are provided ready to use.

All kit components, controls and specimen samples must reach room temperature prior to use. Calibrators, controls, and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. After all kit components have reached room temperature, **mix** gently by inversion.
2. **Prepare** the HRP Conjugate Working Solution and Wash Buffer Working Solution (See section *8. Reagents Provided* section, *2. HRP Conjugate Concentrate* and *8. Wash Buffer Concentrate*).
3. **Prepare** all specimen samples that will be tested. Refer to section *6.2. Specimen Pre-Treatment & Storage.*
4. **Plan** the microplate wells to be used for calibrators, controls, and samples. See section *9. Recommended Assay Layout.* Remove the strips from the microplate frame that will not be used and place them in the bag with desiccant. Reseal the bag with the unused strips and return it to the refrigerator.
5. **Pipette 20 µL** of each calibrator, control, and pre-treated specimen sample into assigned wells.
6. **Pipette 200 µL** of Assay Buffer into each well (the use of a multi-channel pipette is recommended).
7. **Incubate** the microplate on a microplate shaker** for **30 minutes** at room temperature.
8. **Wash** the microplate wells with an automatic microplate washer (preferred) or manually as stated below.

   Automatic: Using an automatic microplate washer, perform a **3-cycle** wash using **300 µL/well** of Wash Buffer Working Solution (3 x 300 µL). One cycle consists of aspirating all wells then filling each well with 300 µL of Wash Buffer Working Solution. After the final wash cycle, aspirate all wells and then tap the microplate firmly against absorbent paper to remove any residual liquid.

   Manually: For manual washing, perform a **3-cycle** wash using **300 µL/well** of Wash Buffer Working Solution (3 x 300 µL). One cycle consists of aspirating all wells by briskly emptying the contents of the wells over a waste container, then pipetting 300 µL of Wash Buffer Working Solution into each well using a multi-channel pipette. After the final wash cycle, aspirate all wells by briskly emptying the contents over a waste container and then tap the microplate firmly against absorbent paper to remove any residual liquid.
9. **Pipette 100 µL** of the HRP Conjugate Working Solution into each well (the use of a multi-channel pipette is recommended).
10. **Incubate** the microplate on a microplate shaker** for **15 minutes** at room temperature.
11. **Wash** the microplate wells again as stated in step 8.
12. **Pipette 100 µL** of TMB Substrate into each well (the use of a multi-channel pipette is recommended).
13. **Incubate** the microplate on a microplate shaker** for **10-15 minutes** at room temperature.
14. **Pipette 50 µL** of Stopping Solution into each well (the use of a multi-channel pipette is recommended) in the same order and speed as was used for addition of the TMB Substrate. Gently tap the microplate frame to mix the contents of the wells.
15. **Measure** the optical density (absorbance) in the microplate wells using an absorbance microplate reader set to 450 nm, within 20 minutes after addition of the Stopping Solution.

** See section *7. Reagents And Equipment Needed But Not Provided* for microplate shaker options.

11. CALCULATIONS

1. Calculate the mean optical density for each calibrator, control and specimen sample duplicate.
2. Use a 4-parameter or 5-parameter curve fit with immunoassay software to generate a calibrator curve.
3. The immunoassay software will calculate the concentrations of the controls and specimen samples using the mean optical density values and the calibrator curve.

   Do not apply any dilution factor to the controls or specimen sample results. The calibrators are provided in a pre-diluted (1:20), ready to use format which automatically compensates for the dilution of specimen samples.
4. If a sample reads more than 10,000 ng/mL and needs to be diluted and retested, then dilute with calibrator A not more than 1:10 from the original 1:20 diluted serum (or 1:200 from neat serum). The result obtained must be multiplied by the dilution factor that was used.

   Example:
   A 1:20 diluted serum sample was further diluted **1:10** in calibrator A and retested. The CRP concentration from the calibrator curve was 20,000 ng/mL.
   The final serum specimen CRP concentration = 20,000 ng/mL x **10** = 200,000 ng/mL.

12. QUALITY CONTROL

When assessing the validity of the test results, the following criteria should be evaluated:

1. The calibrator A mean optical density meets the acceptable range as stated in the QC Certificate.
2. The calibrator with the highest concentration meets the optical density acceptable range as stated in the QC Certificate.
3. The values obtained for the kit controls are within the acceptable ranges as stated in the QC certificate.
4. The results of any external controls that were used meet the acceptable ranges.

13. TYPICAL DATA

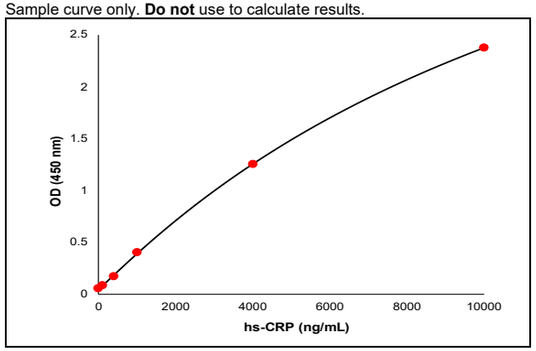
13.1 TYPICAL TABULATED DATA

Sample data only. **Do not** use to calculate results.

Calibrator	Mean OD (450 nm)	% Binding	Value (ng/mL)
A	0.056	2	0
B	0.085	4	100
C	0.174	7	400
D	0.406	17	1000
E	1.254	53	4000
F	2.376	100	10,000
Unknown	1.021	-	3072

13.2 TYPICAL CALIBRATOR CURVE

Sample curve only. **Do not** use to calculate results.



14. SYMBOLS GLOSSARY

Symbol	Definition	Symbol	Definition
REF	Catalogue number		Manufacturer
LOT	Batch code		Date of manufacture
IVD	In vitro diagnostic medical device		Biological risks
UDI	Unique Device Identifier		Consult instructions for use
X #	Dilute 1:# Before Use	Rx ONLY	Prescription only: Device restricted to use by or on the order of a physician
QTY	Quantity		Keep away from sunlight
	Use-by date	EC REP	Authorized representative in the European Community/ European Union
	Do not re-use		Temperature limit
	Caution		Contains sufficient for <n> tests
LYO	Lyophilized	RUO	For Research Use Only. Not for use in diagnostic procedures.
The definitions of symbols used for kit component names are described in the *Reagents Provided* section.			

15. CHANGE HISTORY

Previous Version:	6.0 (Combined)	**New Version:**	RUO-7.0
Changes:	New IFU format with numbered headings. **HEADING** Removal of country-specific regulatory information. Addition of RUO symbol. Removed all performance characteristics, reference ranges and literature/references. **1. INTENDED PURPOSE & USE** Addition: For Research Use Only. Not for use in diagnostic procedures. **2. LIMITATIONS RELATED TO INTENDED PURPOSE & USE** All limitations removed and replaced with: This kit is intended for research use only and is not to be used for any diagnostic procedures. **4. PROCEDURAL CAUTIONS AND WARNINGS** Additional cautions and warnings added. Some previous limitations added to this section. **6.2 Specimen Pre-Treatment & Storage** More detailed instructions provided. **7. REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED** Addition of microplate shaker options. Addition of Polypropylene/HDPE or disposable glass tubes for sample pre-treatment. Addition of vortex mixer. **8. REAGENTS PROVIDED** Addition of symbols for all components and safety information if applicable. In-use stability statement added for all components. Control low and high now called control 1 and 2, respectively. **9. RECOMMENDED ASSAY LAYOUT** New section added. **10. ASSAY PROCEDURE** Component names revised to match symbol definitions. **11. CALCULATIONS** Removed instructions for manually plotting calibrator curve. **12. QUALITY CONTROL** New section added. **13.1 TYPICAL TABULATED DATA** Table data updated. **14. SYMBOLS GLOSSARY** Addition of symbols and definitions. **15. CHANGE HISTORY** New section added. **16. GENERAL INFORMATION** Addition of product complaints, warranty and limitation of liability sections. Build: *v1.4D*		

16. GENERAL INFORMATION

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Product Complaints

In the case of product complaints, the user shall submit in writing to the distributor or manufacturer a description of the complaint and provide accompanying data and/or information.

Warranty

IBL-America guarantees that the product is free of defects and will perform within the product specifications when the product is used prior to the expiration date, according to the intended purpose and use, and according to the instructions for use provided with the product. Any deviations from the intended purpose and use, instructions for use, modifications to kit components or use beyond the expiration date will invalidate any warranty claims.

Limitation of Liability

IBL-America liability in all circumstances whether in tort (including negligence) or at common law, and for any damage or loss, including but not limited to loss of profit and loss of sales, suffered whether direct, indirect, consequential, incidental or special is limited to the purchase price of the product(s) in question.