Version 22-04/24

User's Manual

HIGH SENSITIVITY C-REACTIVE PROTEIN ELISA Kit

REF **DEIA-H015H**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

For the determination of C-Reactive Protein by enzyme immunoassay in human serum.

General Description

C-reactive protein (CRP) is an annular (ring-shaped), pentameric protein found in the blood plasma, the levels of which rise in response to inflammation (i.e., C-reactive protein is an acute-phase protein). Its physiological role is to bind to phosphocholine expressed on the surface of dead or dying cells (and some types of bacteria) in order to activate the complement system via the C1Q complex.

CRP is synthesized by the liver in response to factors released by macrophages and fat cells (adipocytes). It is a member of the pentraxin family of proteins. It is not related to C-peptide (insulin) or protein C (blood coagulation). C-reactive protein was the first pattern recognition receptor (PRR) to be identified.

Principles of Testing

The principle of the following enzyme immunoassay test follows a typical two-step capture or "sandwich" type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for CRP is immobilized onto the microwell plate and another monoclonal antibody specific for a different region of CRP is conjugated to horse radish peroxidase (HRP). CRP from the unknown and calibrators are allowed to bind to the plate, washed, and subsequently incubated with the HRP conjugate. After a second washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microtiter plate reader. The intensity of the colour formed by the enzymatic reaction is directly proportional to the concentration of CRP in the unknown.

A set of calibrators is used to plot a calibration curve from which the amount of CRP in the unknowns being tested and controls can be directly read.

Reagents And Materials Provided

1. Mouse Anti-CRP Antibody Coated Microwell PlateBreak Apart Wells - Ready To Use.

Contents: One 96 well (12x8) monoclonal antibody-coated microwell plate in a resealable pouch with desiccant.

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

2. Mouse Anti-CRP Antibody-Horseradish Peroxidase (HRP) Conjugate Concentrate - Requires Preparation.

Contents: Anti-CRP monoclonal antibody-HRP conjugate in a protein-based buffer with a non-mercury preservative.

Volume: 0.3 mL/vial

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

Preparation: Dilute 1:80 in assay buffer before use (eg. 25 µL of HRP in 2 mL of assay buffer). If the whole plate is to be used dilute 150 µL of HRP in 12 mL of assay buffer. Discard any that is left over.

3. CRP Calibrators - Ready To Use.

Contents: Six vials containing CRP in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of CRP. Calibrated against World Health Organization (WHO) 1st IS 85/506.

*Listed below are approximate concentrations, please refer to vial labels for exact concentrations.

Calibrator	Concentration	Volume/Vial
Calibrator A	0 ng/ml	16 ml
Calibrator B	100 ng/ml	0.5 ml
Calibrator C	400 ng/ml	0.5 ml
Calibrator D	1000 ng/ml	0.5 ml
Calibrator E	4000 ng/ml	0.5 ml
Calibrator F	10,000 ng/ml	0.5 ml

Storage: Refrigerate at 2-8°C

Stability: 12 months in unopened vials or as indicated on label.

Once opened, the calibrators should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

4. Controls - Ready To Use.

Contents: Two vials containing CRP in a protein-based buffer with a non-mercury preservative. Prepared by spiking serum with defined quantities of CRP. Refer to vial labels for the acceptable range.

Volume: 0.5 mL/vial

Storage: Refrigerate at 2-8°C

Stability: 12 months in unopened vial or as indicated on label.

Once opened, the controls should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

5. Wash Buffer Concentrate - Requires Preparation.

Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.

Volume: 50 mL/bottle

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

Preparation: Dilute 1:10 in distilled or deionized water before use. If the whole plate is to be used dilute 50 mL of the wash buffer concentrate in 450 mL of water.

6. Assay Buffer - Ready To Use.

Contents: Two vials containing a protein-based buffer with a non-mercury preservative.

Volume: 40 mL/kit

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

7. TMB Substrate - Ready To Use.

Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.

Volume: 16 mL/bottle

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

8. Stopping Solution - Ready To Use.

Contents: One vial containing 1M sulfuric acid.

Volume: 6 mL/vial

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

Materials Required But Not Supplied

1. Precision pipettes to dispense 10, 20, 50, 100, 190, 200 and 300 µL.
2. Disposable pipette tips.
3. Distilled or deionized water.
4. Plate shaker.
5. Microwell plate reader with a filter set at 450nm and an upper OD limit of 3.0 or greater* (see assay procedure step 13).

Storage

Store the kit at 4°C upon receipt. For more detailed information, please download the following document on our website.

Specimen Collection And Preparation

COLLECTION AND HANDLING OF UNKNOWNS

Approximately 0.1 mL of serum is required per duplicate determination. Collect 4-5 mL of blood into an appropriately labelled tube and allow it to clot. Centrifuge and carefully remove the serum layer. Store at 4°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 unknowns as possible biohazardous materials and take appropriate precautions when handling.

PRETREATMENT OF UNKNOWNS

Dilute serum unknowns 1:20 with calibrator A before use.

Example: To 190 µL of calibrator A add 10 µL of serum (1:20).

*Do not dilute the calibrators and controls, they are ready for use.

Assay Procedure

Pretreatment of Unknowns: Dilute 1:20 With Calibrator A Before Use.

All reagents must reach room temperature before use. Calibrators, controls and unknowns should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. Prepare working solutions of the anti-CRP-HRP conjugate and wash buffer.
2. Remove the required number of microwell strips. Reseal the bag and return any unused strips to the refrigerator.
3. Pipette 20 µL of each calibrator, control and diluted unknown into correspondingly labelled wells in duplicate.
4. Pipette 200 µL of assay buffer into each well (We recommend using a multichannel pipette).
5. Incubate on a plate shaker (approximately 200 rpm) for 30 minutes at room temperature.
6. Wash the wells 3 times with 300 µL of diluted wash buffer per well and tap the plate firmly against absorbent paper to ensure that it is dry (The use of a washer is recommended).
7. Pipette 100 µL of the conjugate working solution into each well (We recommend using a multichannel pipette).
8. Incubate on a plate shaker (approximately 200 rpm) for 15 minutes at room temperature.
9. Wash the wells again in the same manner as step 6.
10. Pipette 100 µL of TMB substrate into each well at timed intervals.
11. Incubate on a plate shaker for 10-15 minutes at room temperature (or until calibrator F attains dark blue colour for desired OD).
12. Pipette 50 µL of stopping solution into each well at the same timed intervals as in step 10.
13. Read the plate on a microwell plate reader at 450nm within 20 minutes after addition of the stopping solution.

* If the OD exceeds the upper limit of detection or if a 450nm filter is unavailable, a 405 or 415nm filter may be substituted. The optical densities will be lower, however, this will not affect the results of controls or unknowns being tested.

Interpretation Of Results

1. Calculate the mean optical density of each calibrator duplicate.
2. Calculate the mean optical density of each unknown duplicate.
3. Subtract the mean absorbance value of the "0" calibrator from the mean absorbance values of the calibrators, controls a

Precautions

1. 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.
2. Control materials or serum pools should be included in every run at a high and low level for assessing the

reliability of results.

3. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
4. In order to reduce exposure to potentially harmful substances, gloves should be worn when handling kit reagents and unknowns.
5. All kit reagents and unknowns should be brought to room temperature and mixed gently but thoroughly before use.

Avoid repeated freezing and thawing of reagents and unknowns.

6. A calibrator curve must be established for every run.
7. The controls should be included in every run and fall within established confidence limits.
8. Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the controls do not reflect established ranges.
9. When reading the microplate, the presence of bubbles in the microwells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.
10. The substrate solution (TMB) 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.
11. When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.
12. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, unknown, calibrator and control.
13. Do not mix various lot numbers of kit components within a test and do not use any component beyond the expiration date printed on the label.
14. Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

SAFETY CAUTIONS AND WARNINGS POTENTIAL BIOHAZARDOUS MATERIAL

Human serum that may be used in the preparation of the calibrators and controls has been tested and found to be nonreactive for Hepatitis B surface antigen and has also been tested for the presence of antibodies to HCV and Human Immunodeficiency Virus (HIV) and found to be negative. However no test method can offer complete assurance that HIV, HCV and Hepatitis B virus or any infectious agents are absent. The reagents should be considered a potential biohazard and handled with the same precautions as applied to any biohazardous material.

CHEMICAL HAZARDS

Avoid contact with reagents containing TMB, hydrogen peroxide and sulfuric acid. If contacted with any of these reagents, wash with plenty of water. TMB is a suspected carcinogen

Limitations

1. All the reagents within the kit are calibrated for the direct determination of CRP in human serum. The kit is not calibrated for the determination of CRP in saliva, plasma or other types of human or animal biologicals.
2. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
3. Any unknowns or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to inaccurate results.
4. Only calibrator A may be used to dilute any high serum unknowns. The use of any other reagent may lead

to false results.

5. The occurrence of heterophilic antibodies in subjects regularly exposed to animals or animal products has the potential of causing interferences in immunological tests. The subject's background, including the frequency of exposure to animals/products, should be considered if false results are suspected.
6. Some subjects may have antibodies to mouse protein that can possibly interfere in this assay. Therefore, the results from any subjects who have received preparation of mouse antibodies should be reviewed with an appropriate degree of consideration.