

**User's Manual**

Human C-Reactive Protein/CRP DuoSet ELISA Kit

REF**DEIA-NS25901**

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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.

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PRODUCT INFORMATION

Intended Use

For the development of sandwich ELISAs to measure natural and recombinant CRP. The Reagent Diluent recommended may be suitable for most cell culture supernate, serum, and plasma samples. The Reagent Diluent selected for use can alter the performance of an immunoassay. Reagent Diluent optimization for samples with complex matrices such as serum and plasma, may improve their performance in this assay.

This kit contains sufficient materials to run ELISAs on 96 well plates, provided the following conditions are met:

1. The reagents are prepared as described in this package insert.
2. The assay is run as described in the General ELISA Protocol.
3. The recommended microplates, buffers, diluents, substrates, and solutions are used.

This package insert must be read in its entirety before using this product. Refer to the Certificate of Analysis for component concentrations as they may vary. For research use only. Not for use in diagnostic procedures.

Reagents And Materials Provided

1. Human C-Reactive Protein/CRP Capture Antibody
2. Human C-Reactive Protein/CRP Detection Antibody
3. Human C-Reactive Protein/CRP Standard
4. Streptavidin-HRP A

Materials Required But Not Supplied

Duo Set ELISA Ancillary Reagent Kits:

Containing 96 well microplates, plate sealers, substrate solution, stop solution, plate coating buffer (PBS), wash buffer, and Reagent Diluent Concentrate

Storage

Store the unopened product at 2 - 8 °C. Do not use past expiration date.

Plate Preparation

1. Dilute the Capture Antibody to the working concentration in PBS without carrier protein. Immediately coat a 96-well microplate with 100µL per well of the diluted Capture Antibody. Seal the plate and incubate overnight at room temperature.
2. Aspirate each well and wash with Wash Buffer, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any

remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.

- Block plates by adding 300 μ L of block buffer to each well. Incubate at room temperature for a minimum of 1 hour.
- Repeat the aspiration/wash as in step 2. The plates are now ready for sample addition.

Reagent Preparation

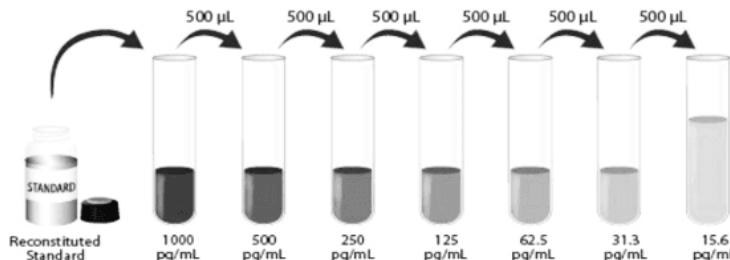
Bring all reagents to room temperature before use. Allow all components to sit for a minimum of 15 minutes with gentle agitation after initial reconstitution. Reconstitute both lyophilized antibodies at the same time. Store the detection antibody at 2-8 °C. Working dilutions should be prepared and used immediately unless otherwise noted.

Streptavidin-HRP A: 1.0 mL of streptavidin conjugated to horseradish-peroxidase. Dilute to the working concentration specified on the vial label using Reagent Diluent.

Human C-Reactive Protein/CRP Capture Antibody: Refer to the lot-specific C of A for amount supplied. Reconstitute with 1.0 mL of PBS. Dilute in PBS without carrier protein to the working concentration indicated on the C of A.

Human C-Reactive Protein/CRP Detection Antibody: Refer to the lot-specific C of A for amount supplied. Reconstitute with 1.0 mL of Reagent Diluent. Dilute in Reagent Diluent to the working concentration indicated on the C of A.

Human C-Reactive Protein/CRP Standard: Refer to the lot-specific C of A for amount supplied. Reconstitute each vial with 0.5 mL of Reagent Diluent. A 7 point standard curve using 2-fold serial dilutions in Reagent Diluent is recommended. Prepare 1000 μ L of high standard per plate assayed at the concentration indicated on the C of A.



Assay Procedure

- Add 100 μ L of sample or standards in Reagent Diluent, or an appropriate diluent, per well. Cover with an adhesive strip and incubate 2 hours at room temperature.
- Repeat the aspiration/wash as in step 2 of the Plate Preparation.
- Add 100 μ L of the Detection Antibody, diluted in Reagent Diluent, to each well. Cover with a new adhesive strip and incubate 2 hours at room temperature.
- Repeat the aspiration/wash as in step 2 of the Plate Preparation.
- Add 100 μ L of the working dilution of Streptavidin-HRP A to each well. Cover the plate and incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
- Repeat the aspiration/wash as in step 2.

7. Add 100 μ L of Substrate Solution to each well. Incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
8. Add 100 μ L of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well immediately, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

Assay Note:

The use of high quality Bovine Serum Albumin (BSA) for the Reagent Diluent and Block Buffer is crucial for the optimum performance of the Duo Set ELISA Development kit. Impurities such as proteases, binding proteins, soluble receptors or other interfering substances can be found to varying degrees in virtually all BSA preparations and can inhibit or interfere with the detection of certain analytes. If the standard curve appears suppressed, consider evaluating a different preparation of BSA.

It is suggested to start Reagent Diluent optimization for serum and plasma samples by using PBS supplemented with 10-50% animal serum. Do not use buffers with animal serum to reconstitute the detection antibody or to dilute the Streptavidin-HRP A.

It is important that the Reagent Diluent selected for dilution of the standard reflects the environment of the samples being measured.

Avoid microbial contamination of reagents and buffers.

A thorough and consistent wash technique is essential for proper assay performance. Wash Buffer should be dispensed forcefully and removed completely from the wells by aspiration or decanting. Remove any remaining Wash Buffer by inverting the plate and blotting it against clean paper towels.

Individual results may vary due to differences in technique, plasticware and water sources.

It is recommended that all standards and samples be assayed in duplicate.

The use of PBS from tablets may interfere in this assay.

Calculation

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

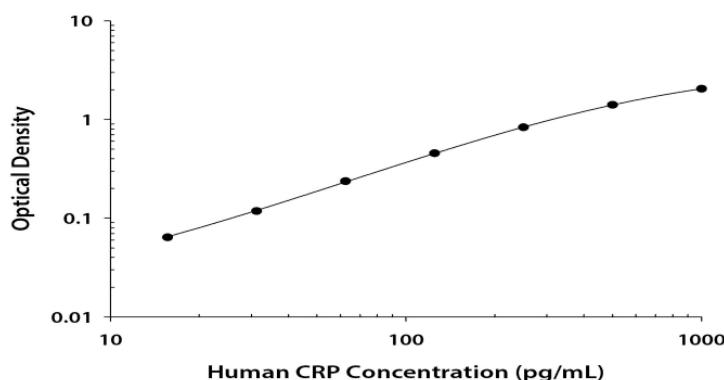
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the CRP concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This

procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Typical Standard Curve

This standard curve is only for demonstration purposes. A standard curve should be generated for each set of

samples assayed.



Detection Range

15.6 - 1,000 pg/mL

Specificity

The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference.

Recombinant human:	Other recombinants:
Pentraxin 2	mouse CRP
Pentraxin 3	porcine CRP
	rat CRP

Precautions

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use. Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist. The Stop Solution suggested for use with this kit is an acid solution.