HUMAN HIGH SENSITIVE C REACTIVE PROTEIN (Hs-CRP) ELISA DEVELOPMENT SET

FOR THE QUANTITATIVE DETERMINATION OF HUMAN Hs-CRP CONCENTRATIONS IN SERUM AND PLASMA

PURCHASE INFORMATION:

ELISA NAME	HUMAN Hs-CRP ELISA DEVELOPMENT SET
Catalog No.	SK00080-07
Lot No.	
Formulation	16 plates
Standard range	78-2500 pg/mL
Sensitivity	50 pg/mL
Sample Volume	100 µl
Sample Type	Serum and Plasma
Specificity	Human CRP only
Intra-assay Precision	8-12%
Inter-assay Precision	12-16%
Storage	2 °C-8 °C

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INTRODUCTION

Human High Sensitive C Reactive Protein ELISA Development Set contains basic components required for the development of sandwich ELISA to measure human CRP in serum and plasma. It contains natural human CRP and antibodies raised against this protein. It has been shown to accurately quantify natural human CRP. Each kit contains sufficient material to run ELISAs on approximately 15 x 96-well plates, provided that the following conditions are met:

- The assay is run as summarized in the Assay Procedure.
- The recommended buffers, diluents, substrates and solutions are used.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for Hs-CRP will be coated onto a microplate. Standards and samples are pipetted into the wells and any CRP present is bound by the immobilized antibody. After washing away any unbound substances, an biotinylated antibody specific for CRP is added to the wells. Following a wash to remove any unbound antibody reagent, Streptavidin HRP conjugate is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of CRP bound in the initial step. The color development is stopped and the intensity of the color is measured.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
Capture Antibody – 0.78 mL/vial of 200-fold concentrated purified antibody against human CRP in PBS, lyophilized	080-07-01	1 vial
CRP Standard – 50 ng/vial of natural human CRP in a buffered protein base with preservatives; lyophilized.	080-07-02	1 vial
Detection Antibody – 0.78 mL/vial of 200-fold concentrated of purified antibody biotinylated against CRP with preservatives; lyophilized.	080-07-03	1 vial
Streptavidin-HRP Conjugate -1.6 mL/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP	SAHRP	1 vial

STORAGE

Unopened Components: Unopened Capture Antibody, Standard and Detection Antibody Store at 2 - 8° C for up to 1 month. For longer storage, unopened Standard, Capture Antibody and Detection Antibody Concentrate should be stored at -20 or -70 °C for up to 6 months. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Aliquot the reconstituted standard, which can be stored for up to 2 months at -70° C. Diluted standard working solution should be prepared and used immediately. Streptavidin - HRP Conjugate 100-fold concentrated may be stored at 2 - 8°C for up to 6 months.

SOLUTIONS REQUIRED

- ***10 x Dilution Buffer required (Aviscera code: DB01A, 50 mL).*** **Note:** DB01A is for high sensitive CRP ELISA development required. This special dilution buffer for standard, sample dilution and detection antibody dilution.
- ***HRP Diluent Solution (DB06) required***. **Note:** DB06 is for Streptavidin HRP conjugate (SAHRP) dilution.
- ***Substrate Solution (Ultra-Sensitive TMB) required***. Aviscera Code: TMB01, 13 ml

- PBS (137 mM NaCl, 2.7 mM KCl, 8.1 mM Na2PO4, 1.5 mM KH2PO4, pH 7.4, 0.2 μm filtered)
- Wash Buffer (0.05% Tween-20 in PBS, pH 7.4)
- Blocking Buffer (BSA base. Code:BS-01)
- STOP SOLUTION (0.5N HCl)
- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

***Note: Wash Buffer, Blocking Buffer, Diluent Solutions (DB01A and DB06), Substrate Solution and Stop Solution are available for purchase online at www.aviscerabioscience.com**

REAGENT PREPARATION

Bring all reagents to room temperature before use.
Capture Antibody - Reconstitute the **Capture Antibody** with 0.78 mL of PBS to produce a 200-fold concentrated stock solution. Pipette 9.95 mL of the appropriate PBS into a 15 mL centrifuge tube and transfer 50 µL of 200-fold concentrated stock solution to prepare working solution. **Note:** Capture Antibody should be diluted without any carry proteins.

CRP Standard - Reconstitute the **CRP** Standard with 1.0 mL of 1 x Diluent Solution (**DB01**). This reconstitution produces a stock solution of 50 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to aliquot. Aliquot and store at -70 °C for up to 2 months. A six point standard curve using 2-fold serial dilutions in Diluent Solution is suggested. A high standard of 2500 pg/ml is recommended.

Detection Antibody - Reconstitute the **Detection Antibody** with 0.78 mL of 1 x Diluent Solution (**DB01**) to produce a 200-fold concentrated stock solution. Pipette 9.95 mL of the Diluent Solution into a 15 mL centrifuge tube and transfer 50 µL of 100-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 9.9 mL of HRP Diluent Solution (**DB06**) into a 15 mL centrifuge tube and transfer 100 µL of 100-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution of Streptavidin-HRP Conjugate should be used within a few days.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that blank, standards and samples be assayed in duplicates.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Coat a 96-well microplate with 100 µL per well of Capture Antibody Working Solution. Seal it and incubate overnight at 2-8 °C.
3. Aspirate each well and wash, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, 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.
4. Add 150 µL per well of Blocking Buffer to each well. Seal plate and incubate for 5 hours at room temperature or overnight at 2-8 °C.
5. Aspirate each well and let it dry.
6. Add 100 µL of standard dilutions and samples per well. Seal plate and incubate for 2 hours on micro-plate shaker at room temperature.
7. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, 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.
8. Add 100 µL of Detection Antibody working solution to each well. Cover with sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
9. Repeat the aspiration/wash as in step 6.
10. Add 100 µL of **Streptavidin-HRP Conjugate working solution** to each well. Incubate for 45 minutes on micro-plate shaker at room temperature. **Protect from light.**
11. Repeat the aspiration/wash as in step 6.
12. Add 100 µL of Substrate Solution to each well. Incubate for 15-20 minutes at room temperature. **Protect from light.**
13. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
14. Determine the optical density of each well within 15 minutes, using a micro-plate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log 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.

Calculation of samples with a concentration exceeding that of standard 2.5 ng/mL may result in inaccurate, low human CRP levels. Such samples require further external predilution according to expected human CRP values with Dilution Buffer in order to precisely quantify the actual human Hs-CRP level.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

STADNARD (PG/ML)	ABSORBANCE 450NM (CORRECTED)
Blank	0 (0.089)
78	0.065
156	0.128
312.5	0.247
625	0.486
1250	0.940
2500	1.702

CALIBRATION

This immunoassay is calibrated against a highly purified natural human CRP.

SPECIFICITY

PROTEIN	CROSSREACTIVITY (%)
Human CRP	100
Human Fetuin A	0
Human VDBP	0
Human MPO	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µL of Capture Antibody working solution to each well. Incubate overnight at 2-8 °C.
↓
Aspirate and wash 4 times.
↓
Add 150 µL of Blocking Buffer to each well. Incubate 4 hours ar RT or overnight at at 2-8 °C.
↓
Aspirate and let it dry.
↓
Add 100 µL of standards and samples to each well. Incubate for 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µL Detection Antibody working solution to each well. Incubate for 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µL Streptavidin HRP conjugate working solution to each well. Incubate 45 min on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 15-20 min on the plate shaker. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min