HUMAN HIGH SENSITIVE C-REACTIVE PROTEIN (hs-CRP) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN C-REACTIVE PROTEIN (CRP) CONCENTRATIONS IN SERUM AND EDTA PLASMA

PURCHASE INFORMATION:

ELISA NAME	HUMAN HIGH SENSITIVE C-REACTIVE PROTEIN (hs-CRP) ELISA
Catalog No.	SK00080-02
Lot No.	
Formulation	96 T
Standard range	39-2500 pg/mL
Sensitivity	15 pg/mL
Sample require	100 µL
Dilution Factor	*10,000 (Optimal dilutions should be determined by each laboratory for each application)*
Sample Type	Serum, EDTA Plasma
Specificity	Human CRP
Intra-assay Precision	4-6%
Inter-assay Precision	8-12%
Storage	2-8 °C

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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INTRODUCTION

Human High Sensitive C-Reactive Protein (CRP) immunoassay is a solid phase ELISA designed to measure human CRP in serum and EDTA plasma. It contains recombinant human CRP and antibodies raised against this protein. It has been shown to accurately quantify recombinant human CRP. Results obtained with naturally occurring CRP samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the immunoassay kit can be used to determine relative mass values for natural human CRP.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for CRP has been pre-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, a biotinylated antibody specific for CRP is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, a 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.

LIMITATIONS OF THE PROCEDURE

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_ The kit should not be used beyond the expiration date on the kit label.

_ Do not mix or substitute reagents with those from other lots or sources.

_ It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.

_ If samples generate values higher than the highest standard, dilute the samples with Dilution Buffer and repeat the assay.

_ Any variation in dilution buffer, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
CRP Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a purified antibody against CRP.	**080-02-01**	**1 plate**
CRP Standard – 10000 pg/vial of recombinant human CRP in a buffered protein base with preservative; lyophilized.	**080-02-02**	**1 vial**
Detection Antibody – 1.2 mL/vial, 10-fold concentrate of a biotinylated antibody against CRP with preservative; lyophilized.	**080-02-03**	**1 vial**
Positive Control – one vial of recombinant human CRP; lyophilized.	**080-02-04**	**1 vial**
Streptavidin HRP Conjugate - 30 µL/vial, 500-fold concentrated solution of Streptavidin HRP conjugate.	**SAHRP**	**1 vial**
Dilution Buffer Concentrate - 50 mL of 10-fold concentrated buffered protein based solution with preservative.	**DB01A**	**1 bottle**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution - 11 mL of TMB substrate solution.	**TMB01**	**1 bottle**
Stop Solution - 11 mL of 0.5M HCl.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**
Plastic Pouch	**P01**	**1 piece**

STORAGE

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

Opened / Reconstituted Reagents: Reconstituted Standard (stock) solution and Detection Antibody concentrated solution (10x-Fold) SHOULD BE STORED at -20 °C or -70 °C for up to one month. Streptavidin-HRP Conjugate 500-fold concentrated

solution **(protect from light)** and other components may be stored at 2 – 8 °C for up to 8 months. Do not freeze TMB substrate solution.
Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack. Microplate may be stored for up to 6 months at 2 – 8 °C after opening.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- PBS
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

PRECAUTIONS FOR USE

All reagents should be considered as potentially hazardous. The stop solution contains diluted hydrochloric acid. Appropriate care, therefore, should be taken while handling this solution.
We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤-20° C. Avoid repeated freeze-thaw cycles.
Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤-20° C. Avoid repeated freeze-thaw cycles.
Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Serum and plasma samples may need a 10,000 fold dilution. A suggested 10,000 fold dilution is 10 µL sample + 990 µL Dilution Buffer to make a 100 fold dilution. Following 10 µL of 100 fold-diluted sample + 990 µL Dilution Buffer to make a 10,000 fold dilution. **Notice:** *CRP concentrations vary greatly, so optimal dilutions should be determined by each laboratory for each application with a pretest.*
Optimal dilutions should be determined by each laboratory for each application.
Use polypropylene test tubes.

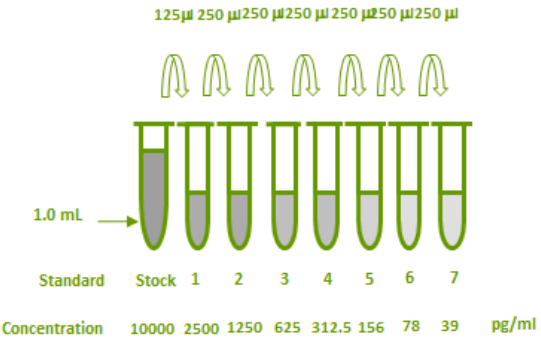
REAGENT PREPARATION

Bring all reagents to room temperature before use.
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of **1x Wash Buffer**.

Dilution Buffer Concentrate (DB01A) - Warm to room temperature. Dilute 50 mL of Dilution Buffer Concentrate into **PBS** (450 mL) to prepare 500 mL of **1x Dilution Buffer**.

CRP Standard - Refer to vial label for reconstitution volume. Reconstitute the **CRP** standard with 1.0 mL of **1x Dilution Buffer**. This reconstitution produces a stock solution of 10000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 375 µL of Dilution Buffer into tubes #1. Transfer 125 µL of 10000 pg/mL stock solution to make 2500pg/mL stock solution. Pipette 250 µL of **1x Dilution Buffer** into tubes #2 to #7. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **2500 pg/mL** standard serves as the high standard. The **1x Dilution Buffer** serves as the zero standard (0 pg/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1 ml	10000 pg/mL
# 1	125µl of stock	375µl	2500 pg/mL
# 2	250µl of 1	250µl	1250 pg/mL
# 3	250µl of 2	250µl	625 pg/mL
# 4	250µl of 3	250µl	312.5 pg/mL
# 5	250µl of 4	250µl	156 pg/mL
# 6	250µl of 5	250µl	78 pg/mL
# 7	250µl of 6	250µl	39 pg/mL



Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with 1.2 mL of **1x Dilution Buffer** to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of **1x Dilution Buffer** into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

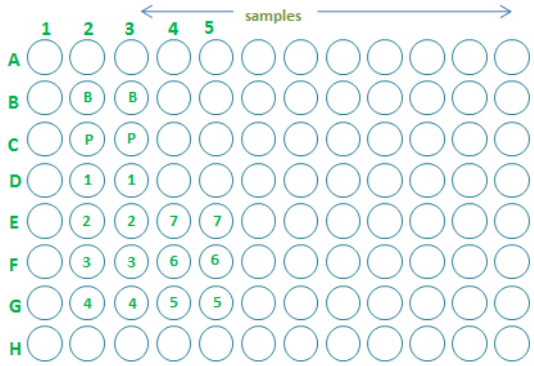
Streptavidin-HRP Conjugate - Transfer 21 µL of 500-fold concentrated Streptavidin-HRP conjugate stock solution to 10.479 mL of **1x Dilution Buffer** to prepare working solution. **Note:** 1x working solution of Streptavidin-HRP Conjugate should be used within a few days. **(PROTECT FROM LIGHT)**

Positive Control - Reconstitute the Positive Control with 1 mL of **1x Dilution Buffer**. **Note:** Positive Control could be reused within a few days if stored at -20 °C or -70 °C.

ASSAY PROCEDURE

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

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Add 100 µL of Dilution Buffer to Blank wells (B2, B3).
4. Add 100 µL of standard solutions from #7 to #1 (reverse order of serial dilution) (from E4, E5 to G4, G5 and G2, G3 to D2, D3), sample, or positive control (C2, C3) per well. Cover with plate sealer. Incubate for 1 hour 30 minutes on microplate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 1x 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.
6. Add 100 µL of Detection Antibody working solution to each well. Cover with plate sealer. Incubate for 1 hour 30 minutes on microplate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Streptavidin-HRP Conjugate working solution to each well. Incubate for 30 minutes on microplate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for 15-20 minutes on microplate shaker at room temperature. **Protect from light.**
11. 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.
12. 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, positive control 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 2500 pg/mL may result in inaccurate, low human CRP levels. Such samples require further external predilution according to expected human CRP values with 1x Dilution Buffer in order to precisely quantify the actual human CRP level.

CALIBRATION

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

SENSITIVITY

The minimum detectable dose (MDD) of CRP was 15 pg/mL.

SPECIFICITY

PROTEIN	CROSS-REACTIVITY
Human CRP	100%
Human PTX3	0
Human Fetuin A	0
Human Gelsolin	0
Human VDBP	0

TYPICAL DATA

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

STANDARD (PG/ML)	AVERAGE OD450 (CORRECTED)
Blank	0 (0.064)
19.5 (optional)	0.019
39	0.034
78	0.069
156	0.138
312.5	0.261
625	0.492
1250	0.945
2500	1.715

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard, samples, positive control to the well. Incubate 1 hour 30 minutes on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Detection Antibody working solution to each well. Incubate 1 hour 30 minutes on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Streptavidin-HRP conjugate working solution to each well. Incubate 30 minutes 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 plate shaker at RT. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min.