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

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

ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

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

PRODUCT INFORMATION:

ELISA NAME	**HUMAN HIGH SENSITIVE C-REACTIVE PROTEIN (CRP) ELISA**
Catalog No.	SK00080-12
Lot No.	
Formulation	96 T
Standard range	4.875 - 625 pg/mL
Sensitivity	4.8 pg/mL
Sample require	100 µL
Dilution Factor	*10,000 ~20,000* ***(Optimal dilutions should be determined by each laboratory for each application)***
Sample Type	Serum, EDTA Plasma
Specificity	Human CRP
Calibration	Human CRP
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 12%
Storage	2 – 8°C
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This High Sensitive C-Reactive Protein (CRP) ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human CRP from serum and EDTA plasma in a sandwich ELISA format.

This immunoassay contains recombinant CRP and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural CRP samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human CRP. The capture antibody can bind to the human CRP in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against CRP is added to the wells. After another washing of the plate, Streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution is added to the wells and color develops in direct proportion to the amount of human CRP bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_This ELISA kit should not be used beyond the expiration date on the kit label.
_Do not mix 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.
_Each laboratory must determine the optimal dilution factors for the samples being assayed with a pretest. If samples generate values that are not within the dynamic range of the standard curve, further concentrate or dilute the samples as required with Dilution Buffer and repeat the assay.
_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.
_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
CRP Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a purified antibody against CRP.	**080-12-01**	**1 plate**
CRP Standard – refer to lot specific of human CRP in a buffered protein base with preservative; lyophilized.	**080-12-02**	**1 vial**
Detection Antibody – refer to lot specific concentrate of a biotinylated antibody against CRP with preservative; lyophilized.	**080-12-03**	**1 vial**
Positive Control – one vial of recombinant human CRP; lyophilized.	**080-12-04**	**1 vial**
Streptavidin HRP Conjugate - 120 µL/vial, 100-fold concentrated solution of Streptavidin HRP conjugate.	**SAHRP**	**1 vial**
Dilution Buffer Concentrate - 30 mL of 10-fold concentrated buffered protein based solution with preservative.	**DB68**	**1 bottle**
HRP Diluent Solution - 12 mL of buffered protein based solution with preservative.	**DB68C**	**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 12 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.

Streptavidin-HRP Conjugate 100-fold concentrated solution **(protect from light)** may be stored at 2 – 8° C for up to 12 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

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.

SAMPLE PREPARATION

Serum and plasma samples may need a 10,000 ~20,000-fold dilution. A suggested 100-fold dilution is 10 µL sample + 990 µL 1x Dilution Buffer, then to make a final 10,000-fold dilution, add 10 µL of 100-fold diluted sample + 990 µL 1x Dilution Buffer. To make a final 20,000-fold dilution, add 150 µL of 10,000-fold diluted sample + 150 µL 1x Dilution Buffer. **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 (DB68) - Warm to room temperature. Dilute 30 mL of Dilution Buffer Concentrate into **deionized water** (270 mL) to prepare 300 mL of **1x Dilution Buffer (DB68)**.

CRP Standard - Reconstitute the CRP standard with refer to lot specific of 1x Dilution Buffer. Pipette 250 µL of 1x Dilution Buffer into tubes #2 to #8. Use the 625 pg/mL high standard solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **625 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	Lot specific	
# 1	125µl of stock	375µl	625 pg/mL
# 2	250µl of 1	250µl	312.5 pg/mL
# 3	250µl of 2	250µl	156.25 pg/mL
# 4	250µl of 3	250µl	78.125 pg/mL
# 5	250µl of 4	250µl	39.063 pg/mL
# 6	250µl of 5	250µl	19.500 pg/mL
# 7	250µl of 6	250µl	9.750 pg/mL
# 8	250µl of 7	250µl	4.875 pg/mL

Positive Control - Reconstitute the Positive Control with refer to lot specific of 1x Dilution Buffer.

Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with refer to lot

specific 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 120 µL of 100-fold concentrated Streptavidin-HRP conjugate stock solution to 11.88 mL of HRP Diluent Solution (DB68C) to prepare working solution. **Note: PROTECT FROM LIGHT.**

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 per well of Dilution Buffer to Blank wells.
4. Add 100 µL per well of standard dilutions from #8 to #1 (reverse order of serial dilution), samples, or positive control. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
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 2 hours 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 60 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 refer to lot specific 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 microplate reader set to 450 nm.

CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log curve fit to more accurately quantify the standard dilutions.

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 625 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.

SPECIFICITY

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

TYPICAL STANDARD CURVE

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

STANDARD (PG/ML)	AVERAGE OD450 (CORRECTED)
Blank	0 (0.047)
4.875	0.035
9.750	0.075
19.500	0.152
39.063	0.288
78.125	0.535
156.250	1.034
312.500	1.745
625.000	2.727

LINEARITY

To assess the linearity of the assay, pooled research human serum samples were diluted with 1x Dilution Buffer DB68 and assayed.

DILUTION FACTOR	ASSAYED (PG/ML)	FINAL (UG/ML)	RECOVERY (%)
10000 X	241.871	2.418	100
20000 X	117.090	2.341	96.8

To assess the linearity of the assay, pooled research human EDTA plasma samples were diluted with 1x Dilution Buffer DB68 and assayed.

DILUTION FACTOR	ASSAYED (PG/ML)	FINAL (UG/ML)	RECOVERY (%)
10000 X	182.439	1.824	100
20000 X	96.795	1.935	106

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard dilutions, samples, or 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 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 60 minutes on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate solution to each well. Incubate refer to lot specific on plate shaker at RT. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min.