Performance characteristics

Intra-assay precision

Samples with known Hu CRP concentration were assayed in replicates of 16 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	51.08	205.01	709.30
SD	3.08	15.35	53.36
%CV	6.02	7.49	7.52

SD = Standard Deviation
CV = Coefficient of Variation

Inter-assay precision

Samples were assayed 48 times in multiple assays to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	56.98	229.33	770.30
SD	5.69	22.51	71.56
%CV	9.98	9.82	9.29

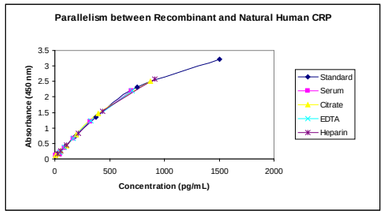
SD = Standard Deviation
CV = Coefficient of Variation

Sensitivity

The minimum detectable concentration of Hu CRP is <10 pg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 28 times, and calculating the corresponding concentration.

Parallelism

Random human serum and plasma samples were serially diluted in the Standard Diluent Buffer. The optical density of each dilution was plotted against the Hu CRP standard curve. Parallelism is shown in the figure below and indicates that the standard accurately reflects the Hu CRP content in natural samples.



Linearity of dilution

Human serum, EDTA plasma, citrate plasma, and heparin plasma spiked with recombinant Hu CRP were serially diluted in Standard Diluent Buffer over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded average correlation coefficients of 0.998 for serum, 0.9988 for EDTA plasma, 0.9973 for citrate plasma, and 0.999 for heparin plasma.

Expected values

Twenty-six random normal serum and plasma samples were evaluated for the presence of Hu CRP in this assay.

Sample	Range (ng/mL)	Average (ng/mL)
Serum (n=12)	52–6203	2109
EDTA plasma (n=5)	354–2337	1259
Citrate plasma (n=4)	150–3046	1664
Heparin plasma (n=5)	357–4207	1662

Specificity

Buffered solutions of a panel of substances ranging in concentrations from 2400–42,000 pg/mL were assayed with Hu CRP kit and found to have no cross–reactivity: Human β2-microglobulin, DR5, Eotaxin, EGF, FGF-basic, G-CSF, Gc-globulin, GM-CSF, Haptoglobin, HGF, IFN-α, IFN-γ, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-15, IL-17, IL-1RA, IL-2R, IL-4R, IP-10, MCP-1, MIP-1α, MIP-1β, MIG, RANTES, SAA, TNF-α, TNF-RI, TNF-RII, and VEGF.

Random, normal serum samples from various species were also evaluated. No cross–reactivity was observed with bovine, goat, hamster, monkey, mouse, rabbit, rat, or swine serum samples.

High dose hook effect

No hook effect was observed with concentrations up to 1 µg/mL.

Limited use label license: Research use only

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Product label explanation of symbols and warnings

	Manufacturer	REF	Catalog Number	LOT	Batch code		Consult instructions for use.		Use by		Temperature limitation		Consult accompanying documents

For support visit thermofisher.com or contact techsupport@lifetech.com.

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24 March 2016

invitrogen PRODUCT INFORMATION SHEET

Human C-Reactive Protein ELISA Kit

Catalog. no. KHA0031 (96 tests)

Pub. No. MAN0004010 Rev 2.00 **Pub. Part no.** PR395

Description

The Human CRP ELISA Kit is a solid-phase sandwich Enzyme Linked Immuno Sorbent Assay (ELISA). This assay is designed to detect and quantify the level of Human C-reactive protein (Human CRP) in serum and plasma. The assay will recognize both natural and recombinant Hu CRP.

C-reactive protein, also known as CRP, is a key acute phase reactant in humans. C-reactive protein was first identified and described by Tillett and Francis in 1930 as a substance present in serum from patients with inflammatory diseases that reacted with the C-polysaccharide of *Streptococcus pneumoniae*. Human C-reactive protein is encoded by a single gene that maps to the first chromosome (1q22–q23). Several genetic polymorphisms in the C-reactive protein gene itself as well as at other loci, are associated with elevated basal C-reactive protein expression levels.

Contents and storage

The components included in the ELISA kit are listed below. Upon receipt, store the kit at 2 to 8°C.

Components	Cat. no. KHA0031 96 tests
Hu CRP Standard, (recombinant Hu CRP), lyophilized, contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume.	2 vials
Standard Diluent Buffer, contains 0.1% sodium azide	2 × 60 mL
Antibody Coated Wells, 12 × 8 Well Strips	1 plate
Hu CRP Biotin Conjugate, (Biotin-labeled anti-CRP), contains 0.1% sodium azide	11 mL
Streptavidin-HRP (100X), contains 3.3 mM thymol	0.125 mL
Streptavidin HRP Diluent, contains 3.3 mM thymol	25 mL
Wash Buffer Concentrate (25X)	100 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB)	25 mL
Stop Solution	25 mL
Plate Covers, adhesive strips	4

CAUTION! This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state, and local regulations for disposal.

Materials required but not provided

- Distilled or deionized water
- Microtiter plate reader (at or near 450 nm) with software
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions

Before starting

Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at thermofisher.com for details prior to starting the procedure.

Note: Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

Note: Allow all reagents to reach room temperature for at least 30 minutes before use.

For research use only. Not for use in diagnostic procedures.

Manufacturing Site • 7335 Executive Way • Frederick • MD 21704 • E-mail: **techsupport@lifetech.com**

Dilute wash buffer

1. Allow the Wash Buffer Concentrate (25X) to reach room temperature and mix to redissolve any precipitated salts.
2. Dilute 1 volume of the Wash Buffer Concentrate (25X) with 24 volumes of deionized water (e.g., 50 mL may be diluted up to 1.25 liters, 100 mL may be diluted up to 2.5 liters). Label as Working Wash Buffer.
3. Store the concentrate and the Working Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

Prepare 1X Streptavidin-HRP solution

Note: Prepare 1X Streptavidin-HRP within 15 minutes of usage.

The Streptavidin-HRP (100X) is in 50% glycerol, which is viscous. To ensure accurate dilution:

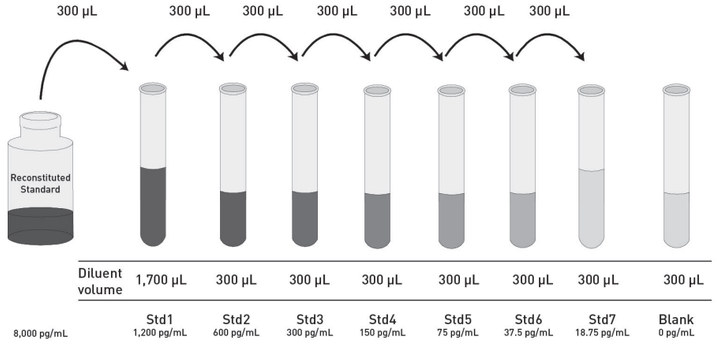
1. For each 8-well strip used in the assay, pipet 10 µL Streptavidin-HRP (100X) solution, wipe the pipette tip with a clean absorbent paper to remove any excess solution, and dispense the solution into a tube containing 990 µL of HRP Diluent. Mix thoroughly.
2. Return the unused Streptavidin-HRP (100X) solution to the refrigerator.

Dilute the standards

Note: This assay has been calibrated against the WHO reference preparation 85/506 (NIBSC, Hertfordshire, UK, EN6 3QG). One nanogram equals 0.98 International Units. Use glass or plastic tubes for diluting standards.

1. Reconstitute Hu CRP Standard to 8000 pg/mL with Standard Diluent Buffer. Refer to the standard vial label for instructions. Swirl or mix gently and allow the contents to sit for 10 minutes to ensure complete reconstitution. Use the standard within 15 minutes of reconstitution. Add 300 µL of the reconstituted standard to a tube containing 1700 µL Standard Diluent Buffer and mix. Label as 1200 pg/mL Hu CRP.
2. Add 300 µL Standard Diluent Buffer to each of 7 tubes labeled as follows: 600, 300, 150, 75, 37.5, 18.75, and 0 pg/mL of Hu CRP.
3. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.

Discard any remaining reconstituted standard. Return the Standard Diluent Buffer to the refrigerator.

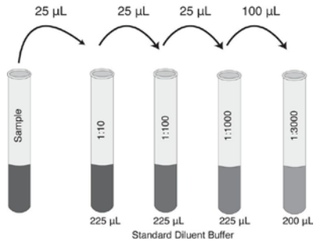


Sample preparation guidelines

- Collect samples in pyrogen/endotoxin-free tubes.
- Samples should be frozen at –80°C if not analyzed shortly after collection. Avoid multiple freeze–thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
- When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

Dilute samples

Human serum and plasma require a 3000–fold dilution in the Standard Diluent Buffer, although further dilutions may be required. A serial dilution of samples is recommended. The suggested dilution series for the 1:3000 dilution is presented below:



ELISA procedure

Allow all reagents to reach room temperature before use. Mix all liquid reagents prior to use. **Total assay time is 4 hours.**

IMPORTANT! Perform a standard curve with each assay.

Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2 to 8°C for future use.

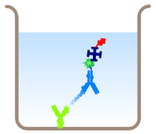
Bind antigen

1. Add 100 µL of Standard Diluent Buffer to the zero standard wells. Wells reserved for the chromogen blank should be left empty. Add 100 µL of standards, diluted samples (see page 2) or controls to the appropriate microtiter wells.
2. Cover the plate with plate cover and incubate for 2 hours at 37°C.
3. Thoroughly aspirate the solution and wash wells 4 times with diluted Wash Buffer.

Add Biotin Conjugate

4. Add 100 µL Hu CRP Biotin Conjugate solution into each well except chromogen blanks.
5. Cover the plate with plate cover and incubate for 1 hour at room temperature.
6. Thoroughly aspirate the solution and wash wells 4 times with diluted Wash Buffer.

Add Streptavidin-HRP



7. Add 100 µL 1X Streptavidin-HRP (see page 2) into each well except the chromogen blanks.
8. Cover the plate with plate cover and incubate for 30 minutes at room temperature.
9. Thoroughly aspirate the solution and wash wells 4 times with diluted Wash Buffer.

Add chromogen

10. Add 100 µL Stabilized Chromogen to each well. The substrate solution will begin to turn blue.
11. Incubate for 30 minutes at room temperature **in the dark**. **Note:** TMB should not touch aluminum foil or other metals.

Add stop solution

12. Add 100 µL Stop Solution to each well. Tap side of the plate gently to mix. The solution in the wells changes from blue to yellow.

Read the plate and generate the standard curve

1. Read the absorbance at 450 nm. Read the plate within 30 minutes after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

   Note: Dilute samples producing signals greater than that of the highest standard in Standard Diluent Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

Standard curve (example)

The following data were obtained for the various standards over the range of 0–1200 pg/mL Hu CRP.

Standard Hu CRP (pg/mL)	Optical density (450 nm)
1200	3.28
600	2.30
300	1.36
150	0.73
75	0.41
37.5	0.23
18.75	0.16
0	0.06