Human C-Reactive Protein ELISA Kit

Catalog Number KHA0031 (96 tests)

Pub. No. MAN0004010 **Rev.** 4.0 (31)

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.

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Invitrogen™ Human C-Reactive Protein ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of human C-Reactive protein in serum and plasma. The assay recognizes both natural and recombinant human C-Reactive protein.

C-reactive protein (CRP), is a key acute phase reactant in humans. The liver is the primary site of C-reactive protein synthesis. According to current models, proinflammatory cytokines produced at localized sites of injury or inflammation, such as IL-6, are transported by the bloodstream to the liver where they modulate the expression of C-reactive protein and other acute phase reactants. Signaling molecules involved in enhanced C-reactive protein expression in response to IL-6 include the gp80 and gp130 components of the IL-6 receptor, and downstream signaling components, including JAKs, STAT3, p38 MAPK, c-jun, and the CAAT enhancer binding protein (C/EBP).

Contents and storage

Upon receipt, store the kit at 2°C to 8°C.

Contents	Cat. No. KHA0031 (96 tests)
Hu CRP Standard, 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 Plate, 96-well strip-well plate	1 plate
Hu CRP Biotin Conjugate; contains 0.1% sodium azide	11 mL
Streptavidin-HRP (100X)	0.150 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

Materials required but not supplied

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

Before you begin

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

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com**.
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Prepare 1X Wash Buffer

1. Dilute 16 mL of Wash Buffer Concentrate (25X) with 384 mL of deionized or distilled water. Label as 1X Wash Buffer.
2. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

Sample preparation guidelines

- Refer to the *ELISA Technical Guide* at **thermofisher.com** for detailed sample preparation procedures.
- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera. If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

Pre-dilute samples

Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

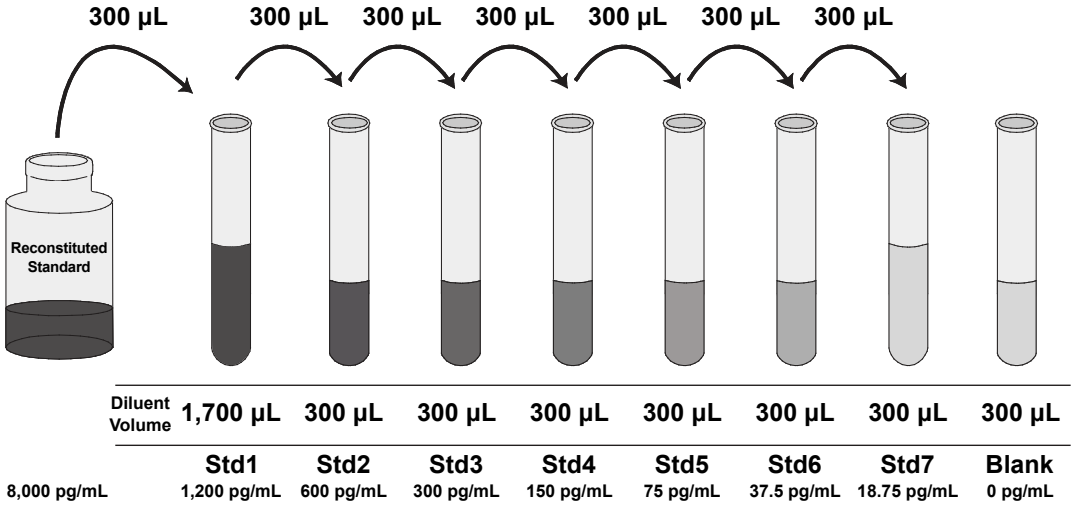
- Perform sample dilutions with Standard Diluent Buffer.
- Dilute serum and plasma samples 1:3,000 with Standard Diluent Buffer.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

This assay has been calibrated against the WHO reference preparation 85/506 (NIBSC, Hertfordshire, UK, EN6 3QG). One nanogram equals 0.98 International Units.

1. Reconstitute Hu CRP Standard to 8,000 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. Label as 8,000 pg/mL human C-Reactive protein. **Use the standard within 15 minutes of reconstitution.**
2. Add 300 µL Reconstituted Standard to one tube containing 1,700 µL Standard Diluent Buffer and mix. Label as 1,200 pg/mL human C-Reactive protein.
3. 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 human C-Reactive protein.
4. Make serial dilutions of the standard as shown in the following dilution diagram. Mix thoroughly between steps.
5. Remaining reconstituted standard should be discarded after completing the assay. Return the Standard Diluent Buffer to the refrigerator.



Prepare 1X Streptavidin-HRP solution

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

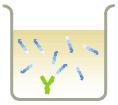
1. For each 8-well strip used in the assay, pipet 10 µL Streptavidin-HRP (100X) solution and dispense the solution into a tube containing 1 mL of Streptavidin-HRP Diluent. Mix thoroughly.
2. Return the unused Streptavidin-HRP (100X) solution to the refrigerator.

Perform ELISA (Total assay time: 4 hours)

IMPORTANT! Perform a standard curve with each assay.

- Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.
- 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°C to 8°C for future use.

1 Bind antigen



a. Add 100 µL of standards, controls, or samples (see "Pre-dilute samples" on page 2) to the appropriate wells. Leave the wells for chromogen blanks empty.
b. Cover the plate with a plate cover and incubate for 2 hours at 37°C.
c. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

2 Add Biotin Conjugate

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

3 Add Streptavidin-HRP

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

4 Add Stabilized Chromogen

a. Add 100 µL Stabilized Chromogen to each well. The substrate solution begins to turn blue.
b. Incubate for 30 minutes at room temperature in the dark.

Note: TMB should not touch aluminum foil or other metals.

5 Add Stop Solution

Add 100 µL Stop Solution to each well. Tap the side of the plate 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 2 hours after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A 4 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 the upper limit of the standard curve in Standard Diluent Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve example

The following data were obtained for the various standards over the range of 0 to 1,200 pg/mL human C-Reactive protein.

Standard Hu CRP (pg/mL)	Optical Density (450 nm)
1,200	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

High-dose hook effect

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

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
Standard Deviation	5.69	22.51	71.56
% Coefficient of Variation	9.98	9.82	9.29

Intra-assay precision

Samples of known human C-Reactive protein concentrations 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
Standard Deviation	3.08	15.35	53.36
% Coefficient of Variation	6.02	7.49	7.52

Expected values

Twenty-six random normal serum and plasma samples were evaluated for the presence of human C-Reactive protein in this assay.

Sample	Range (ng/mL)	Average (ng/mL)
Serum (n=12)	52–6203	2,109
EDTA plasma (n=5)	354–2,337	1,259
Citrate plasma (n=4)	150–3,046	1,664
Heparin plasma (n=5)	357–4,207	1,662

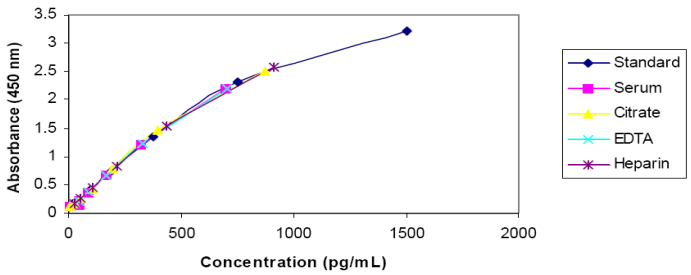
Linearity of dilution

Human serum, EDTA plasma, citrate plasma, and heparin plasma spiked with recombinant human C-Reactive protein 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.

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 human C-Reactive protein standard curve. Parallelism demonstrates that the standard accurately reflects the human C-Reactive protein content in samples.

Parallelism between Recombinant and Natural Human CRP



Sensitivity

The analytical sensitivity of the assay is <10 pg/mL human C-Reactive protein. 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.

Specificity

Buffered solutions of a panel of substances ranging in concentrations from 2,400 to 42,000 pg/mL were assayed with this Human C-Reactive Protein ELISA Kit and found to have no cross-reactivity: **human** β2 microglobulin, DR5, Eotaxin, EGF, FGFbasic, 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.

Limited product warranty

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Product label explanation of symbols and warnings

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

Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria
For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.