Basic CRP Human ELISA Kit

Enzyme-linked immunosorbent assay for quantitative detection of human CRP

Catalog Numbers ECH026 (96 tests)

Pub. No. MAN1000744 Rev. A (30)

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The Basic CRP Human ELISA Kit is an enzyme-linked immunosorbent assay for the quantitative detection of human CRP. This assay is designed to detect and quantify the level of human C-Reactive protein in serum and plasma (EDTA, citrate, and heparin).

C-reactive protein (CRP) is an acute-phase protein produced by the liver in response to inflammation. It is widely used as a clinical biomarker for inflammation and infection. CRP binds to phosphocholine on the surface of dead or dying cells and certain bacteria, activating the complement system and promoting phagocytosis. Elevated levels of CRP are associated with various inflammatory conditions, including cardiovascular diseases, infections, and autoimmune disorders. CRP's role in the acute-phase response makes it a valuable marker for diagnosing and monitoring inflammatory diseases. Additionally, its involvement in atherosclerosis and cardiovascular diseases highlights its potential as a therapeutic target for reducing inflammation and improving cardiovascular health.

For literature updates, go to thermofisher.com.

Contents and storage

- Store kit reagents at 2–8°C.
- Immediately after use, return remaining reagents to cold storage (2–8°C).
- See the expiration date on the package.
- The kit components' expiry is guaranteed only if they are stored properly and not contaminated during repeated use.
- Do not mix components from other lots.

Components	Amount
Coated Microwell Strips	1 pouch (12 strips with 8 wells each)
Biotin-Conjugate (1X)	11 mL
Streptavidin-HRP (100X)	150 µL
Human CRP Standard, lyophilized (8000 pg/mL upon reconstitution)	2 vials
Standard Diluent Buffer	2 × 60 mL
Streptavidin-HRP Diluent; contains 3.3 mM thymol	25 mL
Wash Buffer Concentrate 25X	100 mL
Substrate Solution (Tetramethyl-benzidine)	25 mL
Stop Solution (1M Phosphoric acid)	25 mL
Adhesive Film	6

Required materials not supplied

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at 450 nm (620 nm as optional reference wavelength)
- Beakers, flasks, and cylinders for preparation of reagents
- 5 mL and 10 mL graduated pipettes
- 5 µL to 1000 µL adjustable single channel micropipettes with disposable tips
- 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
- Multichannel micropipette reservoir
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- Statistical calculator with program to perform regression analysis
- Microplate shaker

Before you begin

- Equilibrate the buffer concentrates to room temperature (18–25°C), then dilute before use.
- If crystals have formed in the buffer concentrates, warm gently to dissolve the crystals.

Prepare Wash Buffer (1X)

1. Transfer 16 mL of the Wash Buffer Concentrate (25X) to a clean 500-mL graduated cylinder, then add 384 mL of glass-distilled or deionized water. Mix gently to avoid foaming.
2. Transfer to a clean wash bottle, then label as 1X Wash Buffer.
3. Store Wash Buffer (1X) at 2–8°C for up to 14 days.

Prepare 1X Streptavidin-HRP

Make a 1:100 dilution of the concentrated Streptavidin-HRP Conjugate in a clean plastic tube.

IMPORTANT! Prepare 1X Streptavidin-HRP within 30 minutes of usage.

Dilute 0.12 mL of concentrated Streptavidin-HRP conjugate with 11.88 mL of Assay Buffer (1X), then mix thoroughly.

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.

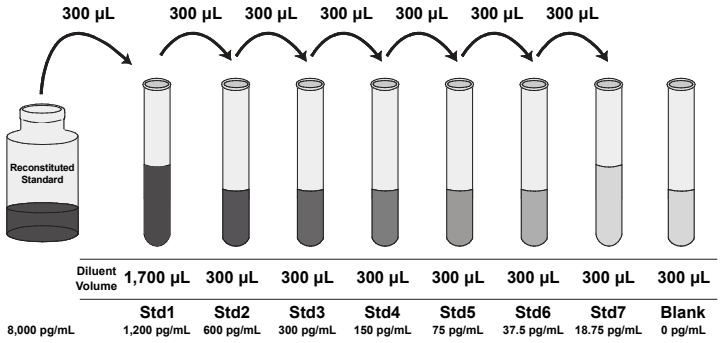
- Perform sample dilutions with Standard Diluent Buffer.
- Dilute serum and plasma samples 1:3,000 with Standard Diluent Buffer.

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.

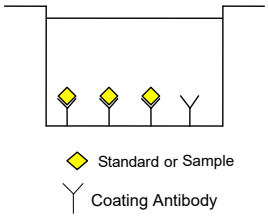
1. Reconstitute Hu CRP Standard to 8,000 pg/mL with Standard Diluent Buffer. Refer to the standard vial label for instructions. Swirl or mix gently and allow the contents to sit for 10 minutes to ensure complete reconstitution. Label as 8,000 pg/mL human C-Reactive protein. **Use the standard within 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.



Perform ELISA protocol

- Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.

1 Bind antigen



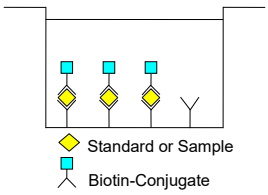
1. Add 100 µL of standards or samples (see “Pre-dilute samples” on page 2) to the appropriate wells. Leave the wells for Chromogen blanks empty.

Table 1 Example of the arrangement of blanks, standards, and samples in the microwell strips

	1	2	3	4	5	6	7	8	9	10	11	12
	Standard		Sample									
A	1	1	1	1	9	9	17	17	25	25	33	33
B	2	2	2	2	10	10	18	18	26	26	34	34
C	3	3	3	3	11	11	19	19	27	27	35	35
D	4	4	4	4	12	12	20	20	28	28	36	36
E	5	5	5	5	13	13	21	21	29	29	37	37
F	6	6	6	6	14	14	22	22	30	30	38	38
G	7	7	7	7	15	15	23	23	31	31	39	39
H	Blank	Blank	8	8	16	16	24	24	32	32	40	40

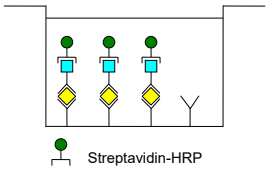
2. Cover the plate with an adhesive film and incubate for 2 hours at 37°C.
3. Remove adhesive film and empty wells. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer. Allow the Wash Buffer to sit in the wells for about 10–15 seconds before aspiration. Do not scratch the surface of the microwells.

2 Add 1X Biotin Conjugate



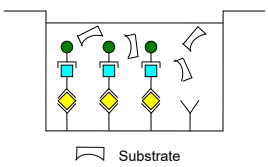
1. Add 100 µL of 1X Biotin-Conjugate solution into each well except the Chromogen blanks.
2. Cover the plate with an adhesive film and incubate for 1 hour at room temperature.
3. Prepare 1X Streptavidin-HRP as mentioned in “Prepare 1X Streptavidin-HRP” on page 2.
4. Remove adhesive film and empty wells. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer. Allow the Wash Buffer to sit in the wells for 10–15 seconds for each wash before aspiration.

3 Add 1X Streptavidin-HRP Conjugate solution



1. Add 100 µL of 1X Streptavidin-HRP Conjugate solution into each well except the Chromogen blanks.
2. Cover the plate with an adhesive film and incubate for 30 minutes at room temperature.
3. Remove adhesive film and empty wells. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer. Allow the Wash Buffer to sit in the wells for 10–15 seconds for each wash before aspiration.

4 Add TMB Substrate Solution (Stabilized Chromogen)

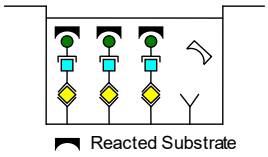


1. Add 100 µL TMB Substrate Solution to all wells.
2. Incubate the microwell strips at room temperature for about 30 minutes. Avoid direct exposure to intense light.

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

   Note: The color development on the plate should be monitored and the substrate reaction stopped (see next step) before positive wells are no longer properly recordable. Determination of the ideal time period for color development has to be done individually for each assay.

5 **Add Stop Solution**



It is recommended to add the stop solution when the highest standard develops a dark blue color.

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.

IMPORTANT! It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme.

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 and unknown samples, prior to plotting.
3. Read the concentrations for unknown samples 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

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 2,400–42,000 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-alpha, IFN-gamma, IL-1 alpha, IL-1 beta, 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-1alpha, MIP-1 beta, MIG, RANTES, SAA, TNF-alpha, 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.

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

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    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and its affiliates warrant their products as set forth in the Life Technologies' General

Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have questions, contact Life Technologies at **www.thermofisher.com/support**.

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN1000744 A (30)

Revision	Date	Description
A (30)	11 December 2024	New document for Basic CRP Human ELISA Kit.

The information in this guide is subject to change without notice.