ab260058 – Human CRP SimpleStep ELISA® Kit (C-Reactive Protein)

For the quantitative measurement of CRP in human serum, plasma, cell culture supernatant, and CSF.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab260058

Storage and Stability: Store kit at 2-8°C immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Standard Preparation and Reagent preparation sections.

Materials Supplied

Item	Quantity 1x96 Tests	Quantity 10x96 Tests	Storage Condition
Human CRP Capture Antibody 10X	600 µL	10 x 600 µL	+4°C
Human CRP Detector Antibody 10X	600 µL	10 x 600 µL	+4°C
Human CRP Lyophilized Recombinant Protein	2 Vials	10 x 2 vials	+4°C
Antibody Diluent 4BI	6 mL	10 x 6 mL	+4°C
Sample Diluent NS	50 mL	2 x 250 mL	+4°C
Wash Buffer PT 10X	20 mL	200 mL	+4°C
TMB Development Solution	12 mL	120 mL	+4°C
Stop Solution	12 mL	120 mL	+4°C
SimpleStep Pre-Coated 96-Well Microplate	96 Wells	10 x 96 Wells	+4°C
Plate Seal	1	10	+4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

Microplate reader capable of measuring absorbance at 450 or 600 nm.
Deionized water.
Multi- and single-channel pipettes.
Tubes for standard dilution.
Plate shaker for all incubation steps.
Hydrochloric acid (HCl)
Sodium Hydroxide (NaOH)
Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.

Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations

1X Wash Buffer PT: Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL Wash Buffer PT 10X with 45 mL deionized water. Mix thoroughly and gently.

Antibody Cocktail: Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 4BI. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent 4BI. Mix thoroughly and gently.

Standard Preparation

Always prepare a fresh set of standards for every use. Discard working standard dilutions after use as they do not store well. The following section describes the preparation of a standard curve for duplicate measurements (recommended).

1. Reconstitute the CRP standard sample by adding the volume of Sample Diluent NS indicated on the protein vial label. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 800 ng/mL **Stock Standard** Solution.

   Acid Treatment Protocol: For dissociation of CRP in standard.
   1) Dilute CRP Stock Standard to 40 ng/mL. Add 5 µL of CRP Stock Standard to 95 µL of Sample Diluent NS.
   2) Add 50 µL of 0.25N HCL to diluted CRP. Incubate for 15 minutes at room temperature while rotating.
   3) Add 50 µL of 0.25N NaOH to acid treated standard. Incubate for 3 minutes at room temperature while rotating.
   4) CRP Stock Standard after treatment is 20 ng/mL; dilute treated CRP Stock Standard in Sample Diluent NS to prepare dilution series.

2. Label eight tubes, Standards 1– 8.
3. Add 376 µL of Sample Diluent NS into tube number 1 and 150 µL of Sample Diluent NS into numbers 2-8.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	**Treated Stock Standard**	24	376	20,000	1,200
2	Standard#1	150	150	1,200	600
3	Standard#2	150	150	600	300
4	Standard#3	150	150	300	150
5	Standard#4	150	150	150	75
6	Standard#5	150	150	75	37.50
7	Standard#6	150	150	37.50	18.75
8	Blank Control	0	150	N/A	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Serum	1:16,000 - 1:1,000
Plasma - Citrate	1:32,000 - 1:2,000
Plasma - EDTA	1:16,000 - 1:1,000
Plasma - Heparin	1:16,000 - 1:1,000
PBMC Cell Culture Supernatant*	< 25%
Cerebrospinal Fluid	0.78 – 12.5%

*Based on spiked sample

Serum Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.

Acid Treatment Protocol: For dissociation of CRP in serum samples.

1) Dilute serum to 10%: Add 10 µL of serum to 90 µL of Sample Diluent NS.
2) Add 50 µL of 0.25N HCL to serum. Incubate for 15 minutes at room temperature while rotating.
3) Add 50 µL of 0.25N NaOH to acid treated sample. Incubate for 3 minutes at room temperature while rotating.
4) Serum concentration after treatment is 5%, dilute treated serum sample in Sample Diluent NS to in assay concentration (see typical dynamic range).

Plasma Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Store un-diluted plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Acid Treatment Protocol: For dissociation of CRP in plasma samples.

1) Dilute plasma to 10%: Add 10 µL of plasma to 90 µL of Sample Diluent NS.
2) Add 50 µL of 0.25N HCL to plasma. Incubate for 15 minutes at room temperature while rotating.
3) Add 50 µL of 0.25N NaOH to acid treated sample. Incubate for 3 minutes at room temperature while rotating.
4) Plasma concentration after treatment is 5%, dilute treated plasma sample in Sample Diluent NS to in assay concentration (see typical dynamic range).

Cell Culture Supernatants Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatant. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Acid Treatment Protocol: For dissociation of CRP in supernatant samples.

1) Add 50 µL of 0.25N HCL to 100 µL neat cell culture supernatant. Incubate for 15 minutes at room temperature while rotating.
2) Add 50 µL of 0.25N NaOH to acid treated sample. Incubate for 3 minutes at room temperature while rotating.
3) Cell culture supernatant concentration after treatment is 50%, dilute treated cell culture supernatant sample in Sample Diluent NS to in assay concentration (see typical dynamic range).

Cerebrospinal Fluid (CSF) Treat cerebrospinal fluid as below and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Acid Treatment Protocol: For dissociation of CRP in cerebrospinal fluid samples.

1) Add 50 µL of 0.25N HCL to 100 µL neat cerebrospinal fluid. Incubate for 15 minutes at room temperature while rotating.
2) Add 50 µL of 0.25N NaOH to acid treated sample. Incubate for 3 minutes at room temperature while rotating.
3) Cerebrospinal fluid concentration after treatment is 50%. Dilute treated cerebrospinal fluid sample in Sample Diluent NS to in assay concentration (see typical dynamic range).

Plate Preparation

The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
For each assay performed, a minimum of two wells must be used as the zero control.
For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
Differences in well absorbance or "edge effects" have not been observed with this assay.

Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use.
We recommend that you assay all standards, controls and samples in duplicate

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.
3. Add 50 µL of all sample or standard to appropriate wells.
4. Add 50 µL of the Antibody Cocktail to each well.
5. Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.
6. Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Wash Buffer PT should remain in wells for at least 10 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and tap gently against clean paper towels to remove excess liquid.
7. Add 100 µL of TMB Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm.
   Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes.
   Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.
8. Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.
9. Alternative to 7 – 8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution

begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode	Kinetic
Wavelength:	600 nm
Time:	up to 20 min
Interval:	20 sec - 1 min
Shaking:	Shake between readings

Note that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
www.abcam.com/protocols/the-complete-elisa-guide

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Additional information

ASSAY SPECIFICITY
This kit is designed for the quantification of human CRP.

The standard protein in this kit is mature full-length human CRP.

Native signal was detected in serum, plasma (heparin), plasma (EDTA), plasma (citrate), and CSF.

Spiked protein experiments were used to validate cell culture supernatant sample types.

Milk, saliva, urine, cell extract, and tissue extract samples have not been tested with this kit.

CROSS REACTIVITY
50 ng/mL of recombinant mouse CRP and 50 ng/mL of recombinant rat CRP were tested for cross reactivity. No cross reactivity was observed.

INTERFERENCE
50 ng/mL of recombinant mouse CRP and 50 ng/mL of recombinant rat CRP were tested for interference. No interference was observed.

SPECIES REACTIVITY
Other species reactivity was determined by measuring 1:1,000 serum samples of various species, interpolating the CRP protein concentrations from the human standard curve, and expressing the interpolated concentrations as a percentage of the CRP protein concentration in human serum assayed at the same dilution.

Reactivity < 3% was determined for the following species: Mouse, Rat, Cow

Other species reactivity not determined.

CALCULATION
- Preconfigured protocols are available when using SoftMax Pro software from Molecular Devices
- Calculate the average fluorescence value for the blank control (zero) standards. Subtract the average blank control standard fluorescence value from all other fluorescence values.
- Create a standard curve by plotting the average blank control subtracted fluorescence value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.
- Note: Most fluorescence reader software or graphing software will plot these values and fit a curve to the data. A four-parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4-parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.
- Determine the concentration of the target protein in the sample by interpolating the blank control subtracted fluorescence values against the standard curve. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.
- Samples generating fluorescence values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at fluorescence values less than that of the lowest standard should be retested in a less dilute form.

TYPICAL DATA
Typical standard curve – data provided for demonstration purposes only. A new standard curve must be generated for each assay performed

Standard Curve Measurements			
Concentration (pg/mL)	O.D. 450 nm		Mean OD
	1	2	
0	0.064	0.058	0.061
18.76	0.125	0.130	0.127
37.50	0.195	0.205	0.200
75.0	0.311	0.329	0.320
150	0.578	0.586	0.582
300	1.076	1.103	1.090
600	2.052	2.070	2.061
1,200	3.395	3.383	3.389

Table 1. Example of human CRP standard curve in Sample Diluent NS. The CRP standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

TYPICAL SAMPLE VALUES

Sensitivity:

The calculated minimal detectable dose (MDD) is 5.36 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=22) and adding 2 standard deviations then extrapolating the corresponding concentration.

Recovery

Three concentrations of CRP were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
1:4,000 Serum	109	105 - 112
1:4,000 Plasma - Citrate	107	105 - 109
1:4,000 Plasma - EDTA	110	107 - 113
1:4,000 Plasma - Heparin	114	112 - 119
25% PBMC Cell Culture Supernatant	100	99 - 100
6.25% Cerebrospinal Fluid	88	80 - 101

Linearity of Dilution

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Native CRP was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Dilution Factor	Interpolated value	1:1,000 Human Serum	1:2,000 Human Plasma (Citrate)	1:1,000 Human Plasma (EDTA)	1:1,000 Human Plasma (Heparin)	12.5% Cerebrospinal Fluid
Undiluted	pg/mL	1,049.0	865.9	707.4	1,102.8	458.1
	% Expected value	100	100	100	100	100
2	pg/mL	526.4	426.1	331.9	551.1	260.9
	% Expected value	100	98	94	100	114
4	pg/mL	253.4	194.2	166.6	263.3	136.1
	% Expected value	97	90	94	96	119
8	pg/mL	125.4	102.3	83.2	126.8	64.8
	% Expected value	96	95	94	92	113
16	pg/mL	63.0	54.9	39.9	65.1	32.4
	% Expected value	96	101	90	94	113

Recombinant CRP was spiked in in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Dilution Factor	Interpolated value	25% PBMC Supernatant
Undiluted	pg/mL	587.8
	% Expected value	100
2	pg/mL	286.6
	% Expected value	98
4	pg/mL	139.9
	% Expected value	95
8	pg/mL	74.2
	% Expected value	101
16	pg/mL	39.9
	% Expected value	109

Precision

Mean coefficient of variations of interpolated values of CRP from a single concentration of human serum within the working range of the assay.

	Intra-assay	Inter-assay
N=	8	3
CV (%)	1.4	4.0

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
www.abcam.com/protocols/the-complete-elisa-guide

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
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