

ab326755 – Dog CRP SimpleStep ELISA® Kit - Chemiluminescent

For the quantitative measurement of CRP in dog serum, plasma (heparin), plasma (EDTA), plasma (citrate), and cell culture supernatant.

For research use only - not intended for diagnostic use.

Patent pending.

For overview, typical data and additional information please visit: www.abcam.com/ab326755

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.

Limitations: All data, except typical standard curve and sensitivity were collected using the colorimetric version of this kit (colorimetric ab256397).

Materials Supplied

Item	Quantity 1 x 96 tests	Quantity 10 x 96 tests	Storage Condition
Dog CRP Capture Antibody 10X	600 µL	10 x 600 µL	+4°C
Dog CRP Detector Antibody 10X	600 µL	10 x 600 µL	+4°C
Dog 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 25BS	20 mL	10 x 20 mL	+4°C
Sample Diluent NS	50 mL	2 x 250 mL	+4°C
Wash Buffer PT 10X	20 mL	200 mL	+4°C
ChemiHRP Reagent A	3 mL	50 mL	+4°C
ChemiHRP Reagent B	3 mL	50 mL	+4°C
SimpleStep Pre-Coated Black 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:

Luminometer capable of reading strip plates with the following settings: 0.5-1 second/well read time; summation mode (all wavelengths).

Deionized water.

Multi- and single-channel pipettes.

Tubes for standard dilution.

Orbital microplate shaker for all incubation steps: capable of 750 rpm shaking speed.

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.

Sample Diluent 25BS may contain precipitate, this is normal. If precipitate is not dissolved by gentle mixing, the precipitate may be dissolved by gentle warming and mixing at 37°C for 10 minutes. If precipitate remains, gently spin down and avoid visible precipitates when pipetting.

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.

Lumi HRP Development Solution: Just prior to use, prepare Lumi HRP Development Solution by mixing equal volume of the ChemiHRP Reagent A and the ChemiHRP Reagent B. To make 3 mL of the Lumi HRP Development Solution combine 1.5 mL of ChemiHRP Reagent A and 1.5 mL of ChemiHRP Reagent B. Mix thoroughly and gently by inversion or slow pipetting (Avoid shaking or vortexing). Protect the prepared solution from light until use.

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** protein standard by adding the volume indicated on the protein vial label. For **cell culture supernatants samples measurements**, use Sample Diluent NS. Hold at room temperature for 10 minutes and mix thoroughly and gently. This is the 5,120 ng/mL **Stock Standard** Solution.
2. Label nine tubes, Standards 1– 9.
3. Use the same Sample Diluent as used to resuspend the Stock Standard to prepare the standard curve. For **cell culture supernatants samples measurements**, add 487 µL of Sample Diluent NS into tube number 1 and 150 µL of Sample Diluent into numbers 2-9.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #9 contains no protein and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (ng/mL)	Final Conc. (ng/mL)
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1	Stock Standard	25	487	5,120	250
2	Standard#1	75	150	250	83.3
3	Standard#2	75	150	83.3	27.8
4	Standard#3	75	150	27.8	9.26
5	Standard#4	75	150	9.26	3.09
6	Standard#5	75	150	3.09	1.03
7	Standard#6	75	150	1.03	0.34
8	Standard#7	75	150	0.34	0.11
9	Blank Control	0	150	0	0

1. Reconstitute the **CRP** protein standard by adding the volume indicated on the protein vial label. For **serum and plasma samples measurements**, use Sample Diluent 25BS. Hold at room temperature for 10 minutes and mix thoroughly and gently. This is the 5,120 ng/mL **Stock Standard** Solution.
2. Label nine tubes, Standards 1–9.
3. For serum and plasma samples measurements, add 375 µL of Sample Diluent 25BS into tube number 1 and 150 µL of Sample Diluent into numbers 2-9.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #9 contains no protein and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (ng/mL)	Final Conc. (ng/mL)
1	Stock Standard	25	375	5,120	320
2	Standard#1	75	150	320	106.7
3	Standard#2	75	150	106.7	35.6
4	Standard#3	75	150	35.6	11.9
5	Standard#4	75	150	11.9	3.95
6	Standard#5	75	150	3.95	1.32
7	Standard#6	75	150	1.32	0.44
8	Standard#7	75	150	0.44	0.15
9	Blank Control	0	150	0	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	Range

Serum	6.25 - 50%
Plasma – Citrate	1.56 - 50%
Plasma – EDTA	12.5 - 50%
Plasma – Heparin	0.78 - 25%
RPMI 1640 Cell Culture Media*	6.25 - 100%

*Media is RPMI 1640 containing 10% fetal bovine serum.

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. Dilute samples at least 1:2 into Sample Diluent 25BS and assay. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.

Plasma Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Dilute plasma-citrate and plasma-EDTA samples at least 1:2 into Sample Diluent 25BS and assay. Dilute plasma-heparin samples at least 1:4 into Sample Diluent 25BS and assay. Store un-diluted plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Cell Culture Supernatants Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants. Assay, or dilute samples into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

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 30 minutes at room temperature on a plate shaker set to 750 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 30 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 50 µL of prepared Lumi HRP Development Solution to each well and incubate for 1 minute in the dark on a plate shaker set to 750 rpm. Further optimization of incubation time vs signal strength can be performed if needed. Avoid introducing bubbles into the wells.
8. Measure the produced light of each well using a microplate luminometer with the following settings: 0.5-1 second/well read time in summation mode (all wavelengths). Relative light unit (RLU) readings may vary between luminometer models. It is recommended to configure instrument settings according to the manufacturer's specifications. Note: Relative light unit (RLU) values may change over the course of the 15-minute reading window.
9. Analyze the data as described below.

Mode:	Luminescence
Instrument settings:	Endpoint
Detection Mode:	All wavelengths
Read Time:	0.5-1 sec
Read:	Top

Note For microplate readers with Pre-Read Optimization option, the Read Height as well as Microplate Optimization is recommended before the first read.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:

<https://www.abcam.com/en-us/technical-resources/guides/elisa-guide>

Technical Support

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Additional information

ASSAY SPECIFICITY

This kit is designed for the quantification of dog CRP.

The standard protein in this kit is the full length mature dog CRP.

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

Spiked protein experiments were used to validate serum, plasma (heparin), and cell culture media.

Urine, saliva, milk, CSF, cell and tissue extract samples have not been tested with this kit.

SPECIES REACTIVITY

Other species reactivity was determined by measuring 50% serum samples of various species, interpolating the protein concentrations from the dog standard curve, and expressing the interpolated concentrations as a percentage of the protein concentration in dog serum assayed at the same dilution.

Interpolated values were below the detectable range of the assay for the following species: Human, Monkey, Mouse, Rat, Cow

Other species reactivity not determined.

CALCULATION

- Preconfigured protocols are available when using SoftMax Pro software from Molecular Devices.
- Calculate the average chemiluminescence value for the blank control (zero) standards. Subtract the average blank control standard chemiluminescence value from all other chemiluminescence values.
- Create a standard curve by plotting the average blank control subtracted chemiluminescence 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 chemiluminescence 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 chemiluminescence 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 chemiluminescence values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at chemiluminescence 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 (ng/mL)	RLU		Mean RLU
	1	2	
0.00	581	647	614
0.11	1,112	1,070	1,091
0.34	2,143	2,063	2,103
1.03	4,634	4,915	4,775
3.09	15,485	15,828	15,657
9.26	51,758	50,299	51,029
27.8	187,240	183,870	185,555
83.3	544,250	544,040	544,145
250	1,389,900	1,560,300	1,475,100

Table 1. Example of dog 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.

Standard Curve Measurements			
Concentration (ng/mL)	RLU		Mean RLU
	1	2	
0.00	530	615	573
0.15	884	869	877
0.44	1,865	1,798	1,832
1.32	5,614	5,865	5,740
3.95	12,167	13,551	12,859
11.9	45,164	44,518	44,841
35.6	115,480	119,880	117,680
106.7	344,010	330,770	337,390
320	1,033,400	1,042,100	1,037,750

Table 2. Example of dog CRP standard curve in Sample Diluent 25BS. The CRP standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

TYPICAL SAMPLE VALUES

Sensitivity:

The minimal detectable dose (MDD) was determined by calculating the mean of zero standard replicates and adding 2 standard deviations then extrapolating the corresponding concentration.

Sample Diluent Buffer	N=	Minimal Detectable Dose
Sample Diluent NS	16	0.07 ng/mL
Sample Diluent 25BS	16	0.10 ng/mL

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 (%)
25% Serum	99	98 - 100
25% Plasma - Citrate	87	81 - 92
25% Plasma - Heparin	102	101 - 102
10% Plasma - EDTA	83	83 - 84
100% RPMI 1640 Cell Culture Media	115	106 - 122

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 dog plasma (citrate) and plasma (EDTA) biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent 25BS.

Recombinant CRP was spiked into dog serum, plasma (heparin), and RPMI 1640 cell culture media containing 10% FBS samples and diluted in a 2-fold dilution series. Serum and plasma (heparin) sample dilutions are made in Sample Diluent 25BS. RPMI 1640 media sample dilutions are made in Sample Diluent NS.

Dilution Factor	Interpolated value	50% Dog Serum*	50% Dog Plasma (Citrate)	25% Dog Plasma (EDTA)	25% Dog Plasma (Heparin)*	100% RPMI Media
Undiluted	ng/mL	239.1	142.3	305.6	218.7	260.7
	% Expected value	100	100	100	100	100
2	ng/mL	109.4	73.79	142.2	96.68	126.0
	% Expected value	91	104	93	88	97
4	ng/mL	55.41	37.77	68.43	46.72	55.16
	% Expected value	93	106	90	85	85
8	ng/mL	27.76	17.60	32.65	25.12	26.42
	% Expected value	93	99	85	92	81
16	ng/mL	13.79	9.641	16.65	11.96	12.87
	% Expected value	92	108	87	88	79

*Native serum and plasma (heparin) values are shown in the linearity graph and legend.

Precision

Mean coefficient of variations of interpolated values of CRP from two concentration of dog plasma (EDTA) within the working range of the assay.

	Intra-assay	Inter-assay
N=	8	3
CV (%)	3.4	1.0

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