Speedy™ Rat C-Reactive Protein/CRP One-Step ELISA Kit Datasheet

Please read it entirely before use

Catalogue Number: SE70005

Size: **96T**

Sensitivity: 10.2 pg/mL

Range: 39-2500 pg/mL

Usage: For the quantitative detection of rat C-Reactive Protein/CRP concentrations in serum, plasma, cell culture supernatant, urine and tissue lysate.

This product is for research use only and not for use in human or animal therapeutic or diagnostic.

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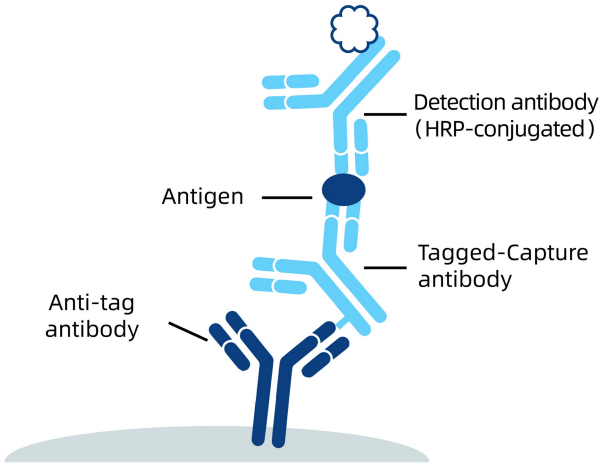
1. Background

C-reactive protein (CRP) is an evolutionary highly conserved member of the pentraxin superfamily of proteins. CRP consists of five identical subunits with molecular weight 20-30 kDa arranged as a cyclic pentamer. CRP exists in at least two conformationally distinct forms, native pentameric CRP (pCRP) and modified/monomeric CRP (mCRP). CRP plays important roles in inflammatory processes and host responses to infection including promoting agglutination, bacterial capsular swelling, phagocytosis, and complement fixation through its Ca2+-dependent binding to phosphorylcholine (PCh). CRP is mainly expressed by hepatocytes and secreted into plasma. The concentration of CRP will increase significantly in plasma during acute phase response to tissue injury, infection, or other inflammatory stimuli.

2. Principle



An anti-tag antibody is pre-coated onto the bottom of wells. After adding antigen or samples, Tagged-Capture antibody and HRP-conjugated detection antibody, a sandwich complex is formed in the solution. TMB acts as a HRP substrate, and the solution color will change from colorless to blue. A stop solution containing sulfuric acid turns the solution yellow. The color intensity is proportional to the quantity of bound protein, which is measurable at 450 nm with the correction wavelength set at 630 nm.

3. Required Materials

3.1 A microplate reader capable of measuring absorbance at 450 nm with the correction wavelength set at 630 nm.

3.2 Calibrated, adjustable precision pipettes and disposable plastic tips. A manifold multi-channel pipette is recommended for large assays.

3.3 Plate washer: automated or manual.

3.4 Absorbent paper towels.

3.5 Glass or plastic tubes to prepare standard and sample dilutions.

3.6 Beakers and graduated cylinders.

3.7 Log-log or semi-log graph paper or computer and software for ELISA data analysis. A four-parameter logistic (4-PL) curve-fit is recommended. Proteintech data analysis website, https: //www.ptgcn.com/products/elisa-kits/.

3.8 Microplate thermostatic shaker.

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4. Kit Components and Storage

Microplate - 96 well microplate precoated an anti-tag antibody (8 well × 12 strips)	1 plate	**Unopened Kit:** Store at 2-8°C for 6 months or -20°C for 12 months. **Opened Kit:** All reagents stored at 2-8°C for 7 days. **Please use a new standard for each assay.**
Protein standard - 5000 pg/bottle; lyophilized	2 bottles	
Capture antibody (100×) - 60 uL/vial*	1 vial	
Detection antibody, HRP-Conjugated (100×) - 60 uL/vial*	1 vial	
Sample Diluent PT 4B1 - 30 mL/bottle	2 bottles	
Detection Diluent - 15 mL/bottle	1 bottle	
Wash Buffer Concentrate (20×) - 30 mL/bottle	1 bottle	
Extraction Reagent - 15 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	4 pieces	

* Centrifugation immediately before use

5. Safety Notes

5.1 Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

5.2 Do not use the kit after the expiration date.

5.3 Do not mix or substitute reagents or materials from other kit lots or other sources.

5.4 Be sure to wear protective equipment such as gloves, masks and goggles during the experiment.

5.5 When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer to improve assay precision

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6. Sample Collection and Storage

6.1 Serum: Allow blood samples to clot for 30 minutes, followed by centrifugation for 15 minutes at 1000xg. Clear serum can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.

6.2 Plasma: Use EDTA, heparin, or citrate as an anticoagulant for plasma collection. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. The plasma can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.

6.3 Cell Culture Supernatant: Remove particulates by centrifugation for 5 minutes at 500xg and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

6.4 Urine: Collect urine samples and centrifuge for 20 minutes at 1000xg. Collect the aqueous layer, assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

6.5 Tissue Lysate:

1) Rinse tissue with PBS, cut into 1-2 mm pieces.

2) Add protease inhibitor cocktail to the Extraction Reagent to a final concentration immediately prior to performing tissue lysis.

3) Add 1 mL of Extraction Reagent containing protease inhibitor cocktail per 100 mg tissue.

4) Homogenize the tissue completely using desired method on ice, Incubate on ice for 30 minutes, use ultrasound to break up the cells.

5) Centrifuge tissue homogenates at 10,000 x g for 5 minutes at 4°C. Collect the supernatant, assay immediately or aliquot and store at -20°C.

6) Measure the concentration of total protein in tissue homogenates using BCA assay.

7) Avoid protein degradation by performing all the above procedures on ice where possible.

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7. Reagent Preparation

7.1 Wash Buffer (1X): If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 30 mL of Wash Buffer Concentrate(20X) to 570 mL deionized or distilled water to prepare 1X Wash Buffer.

7.2 Antibody Cocktail (1X): Dilute 100X capture antibody and 100X HRP-conjugated detection antibody using Detection Diluent prior to assay. Suggested 1:100 dilution: 50 uL 100X capture antibody + 50 uL 100X Detection Antibody, HRP-conjugated + 4,900 uL Detection Diluent. Mix gently but thoroughly.

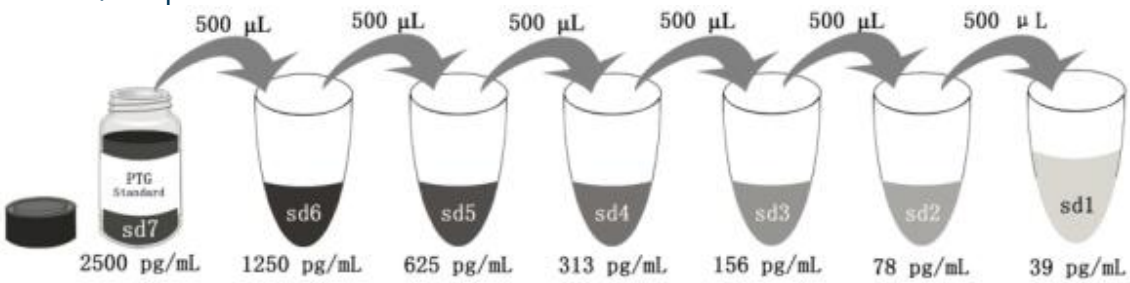
7.3 Sample Dilution: Different samples should be diluted with corresponding Sample Diluent. The minimum required dilution factor was 1:2; consequently, no more than 25 uL of undiluted sample was added per well.

Samples may require further dilution if the readout values are higher than the highest standard OD reading. Variations in sample collection, processing and storage may affect the results of the measurement.

Recommended Dilution for different sample types: 1:800,000 or 1:1,600,000 is recommended for rat serum and plasma; 1:2 to 1:200 is recommended for cell culture supernatant; 1:640 or 1:1280 is recommended for urine; 1:2,000 or 1:4,000 is recommended for tissue lysate.

7.4 Standard Serial Dilution:

Add 2 mL Sample Diluent PT 4B1 in protein standard.



Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 4B1	**2000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

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8. Assay Procedure Summary

Bring all reagents to room temperature before use (Detection antibody, HRP-conjugated antibody can be used immediately). To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

8.1 Preset the layout of the microplate, including control group, standard group and sample group, take out the required number of microplate strips and return excess strips to the foil pouch containing the drying reagent pack and reseal; store at 4°C immediately. Microplate strips should be used in one week.

8.2 Add 50 μL standard or sample to appropriate wells. To avoid high background always add samples or standards to the well before the addition of antibody cocktail.

8.3 Add 50 μL 1× Antibody Cocktail solution (refer to Reagent Preparation 7.2) to each well. Seal plate with cover seal and incubate at 37°C on a microplate thermostatic shaker set at 400 rpm for 1 hour (incubate at 37°C for 2 hours is recommended if thermostatic shaker is not available) .

8.4 Wash

1) Gently remove the cover seal. Discard the liquid from wells by aspirating or decanting. Remove any residual solution by tapping the plate a few times on fresh paper towels.

2) Wash 4 times with 1× Wash Buffer, using at least 350-400 μL per well. Following the last wash, firmly tap plates on fresh towels 10 times to remove residual Wash Buffer. Avoid getting any towel fibers in the wells or wells drying out completely.

8.5 Add 100 μL TMB substrate solution to each well, protected from light. Incubate at 37°C on a microplate thermostatic shaker set at 400 rpm for 15 to 20 minutes. (Substrate Solution should remain colorless until added to the plate.)

8.6 Add 100 μL Stop Solution to each well in the same order as addition of the TMB substrate. Note: Avoid skin and eye contact with the Stop solution.

8.7 Read results immediately on a microplate reader at a wavelength of 450 nm. If possible, perform a double wavelength readout (450 nm and 630 nm).

8.8 Data analysis: Calculate the average of the duplicate readings (OD value) for each standard and sample, and subtract the average of the zero standard absorbance. Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis, four-parameter logistic curve- fit (4-PL) analysis is recommend. If the samples have been diluted, the fitting result must be multiplied by the dilution factor used.

Procedure summary

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Add 50 µL standard or sample to appropriate wells

Add 50 µL antibody cocktail solution (1x) to each well

Incubate at 37 °C on a microplate thermostatic shaker set at 400 rpm for 1 hour

Aspirate and wash each well four times with 400µL 1 x Wash Buffer

Add 100 µL TMB substrate solution to each well

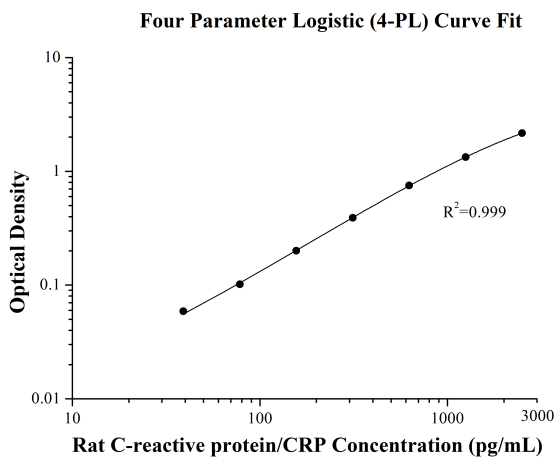
Incubate at 37 °C on a microplate thermostatic shaker set at 400 rpm for 15minutes

Add 100 µL Stop Solution to each well and Read OD immediately

9. Validation Data

9.1 Standard curve

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D	Average	Corrected
0	0.0304 0.0276	0.029	-
39	0.0928 0.0827	0.08775	0.05875
78	0.1288 0.1326	0.1307	0.1017
156	0.2327 0.2264	0.22955	0.20055
313	0.4237 0.4167	0.4202	0.3912
625	0.7877 0.7718	0.77975	0.75075
1250	1.3288 1.3951	1.36195	1.33295
2500	2.2404 2.1546	2.1975	2.1685

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9.2 Precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 8 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 16 separate assays to assess inter-assay precision.

Intra-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	8	1,344.6	57.2	4.3
2	8	338.2	5.1	1.5
3	8	188.8	4.5	2.4

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	16	1,327.6	65.2	4.9
2	16	339.5	8.2	2.4
3	16	187.6	5.7	3.0

9.3 Recovery

The recovery of rat C-Reactive Protein/CRP spiked to three different levels throughout the range of the assay in various matrices was evaluated.

Sample Type		Average% of Expected	Range (%)
Rat serum	1:3,200,000	102	99-107
	1:6,400,000	105	99-111
Cell culture supernatant	1:16	118	107-127
	1:32	95	93-97
Urine	1:2,560	118	112-123
	1:5,120	114	105-127
TIssue lysate	1:8,000	91	89-93
	1:16,000	93	92-94

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9.4 Sample values

Rat serum/Urine - Rat serum and urine samples were evaluated for the presence of rat C-Reactive Protein/CRP in this assay.

Sample Type	Mean (**µg/mL**)	Range (**µg/mL**)
Rat serum (n=16)	548.2	328.8-730.7

Sample Type	Mean (**ng/mL**)	Range (**ng/mL**)
Urine (n=8)	257.3	36.2-891.0

Cell culture supernatant:

Rat heart tissue was collected after culturing for 4 days in RPMI with 10% fetal bovine serum. An aliquot of the cell culture supernate was removed, assayed for rat C-Reactive Protein/CRP, and measured 285.1 **ng/mL**.

Rat spleen tissue was collected after culturing for 2 days in RPMI with 10% fetal bovine serum. An aliquot of the cell culture supernate was removed, assayed for rat C-Reactive Protein/CRP, and measured 1,368.0 pg/mL.

Tissue lysate

	Rat C-Reactive Protein/CRP (**µg/mL**)	Total protein (**mg/mL**)
Rat heart tissue lysate	81.2	6.7
Rat spleen tissue lysate	39.0	8.8

9.5 Sensitivity

The minimum detectable dose of rat C-Reactive Protein/CRP is 10.2 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

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9.6 Linearity

To assess the linearity of the assay, samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

(The rat serum was initially diluted 1:400,000. The cell culture supernatant was initially diluted 1:100. The urine was initially diluted 1:320. The tissue lysate was initially diluted 1:1,000.)

		Rat serum	Cell culture supernatant	Urine	Tissue lysate
1:2	Average% of Expected	100	100	100	100
	Range (%)	-	-	-	-
1:4	Average% of Expected	98	98	92	95
	Range (%)	96-100	96-99	91-93	89-102
1:8	Average% of Expected	100	100	88	98
	Range (%)	99-101	97-102	86-89	85-111
1:16	Average% of Expected	97	99	86	93
	Range (%)	91-103	93-105	85-87	83-103

9.7 Specificity

This assay recognizes natural and recombinant rat C-Reactive Protein/CRP.

The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference.

Recombinant human:	Recombinant mouse:
CRP	CRP

10. References

1. Xinrong Wang. et al. (2020) Front Cell Dev Biol. 8:599281.
2. Michael Simons. et al. (2016) Nat Rev Mol Cell Biol. 17(10):611-25.
3. Jing Yu. et al. (2016)J Hematol Oncol. 9(1):111.
4. Seong Ah Park. et al. (2018) BMB Rep. 51(2):73-78.

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