PRODUCT INFORMATION & MANUAL

Human C-Reactive Protein (CRP) Valukine^{TM} ELISA

Catalog Number: **VAL120**

For the quantitative determination of natural and recombinant
human C-Reactive Protein (CRP) concentrations

For research use only.
Not for diagnostic or therapeutic procedures.

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Please refer to the kit label for expiry date.
Novus kits are guaranteed for 3 months from date of receipt

Version 202308.3

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I. BACKGROUND

C-Reactive Protein (CRP), also known as Pentraxin 1, is a non-glycosylated protein in the Pentraxin family that also includes Pentraxin 2/SAP and Pentraxin 3/TSG-14. CRP functions as a sensor and activator for the innate immune response (1). In humans, it is a major acute-phase protein; its circulating concentration is dramatically elevated at the onset of inflammation (2). In mice, however, serum CRP levels increase only slightly during inflammation, and the analogous acute phase role is filled by Pentraxin 2 (3). CRP binds, opsonizes, and induces the phagocytosis of bacteria and apoptotic cells (4, 6-8). It regulates activation of the classical complement pathway by binding several proteins in the complement cascade as well as Fc gamma RI, Fc gamma RIIA, and Fc gamma RIIB on macrophages and dendritic cells (6, 7, 9-11, 12-14). It also promotes dendritic cell maturation and humoral immunity (8). In cardiovascular disease, CRP binds to oxidized LDL, exacerbates tissue damage in myocardial infarction, and inhibits the repair of injured vascular endothelium (5, 15, 16).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human CRP has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any CRP present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated detection antibody specific for human CRP is pipetted into the wells. After washing away any unbound substances, Streptavidin-HRP is pipetted into the wells. Following a wash to remove any unbound reagent, TMB substrate solution (Chromogenic agent) is added to the wells and color develops in proportion to the amount of CRP bound in the initial step. The color development is stopped, and the intensity of the color is measured.

B. LIMITATIONS OF THE PROCEDURE

- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**
- This kit is suitable for cell culture supernate and human serum.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with Reagent Diluent (1×) and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

III. ADVANTAGES

A. PRECISION

Intra-assay Precision (Precision within an assay)

Three samples were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)

Three samples were tested in twenty separate assays to assess inter-assay precision.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
Mean (pg/mL)	52.3	207.5	854.4	51.7	204.3	874.0
Standard Deviation	1.3	9.6	46.7	1.5	8.0	55.2
CV%	2.6	4.6	5.5	3.0	3.9	6.3

B. RECOVERY

The recovery of human CRP spiked to three levels throughout the range of the assay in cell culture media was evaluated. The recovery ranged from 84.1 to 87.8% with an average of 86.9%.

C. SENSITIVITY

The minimum detectable dose (MDD) of CRP is typically less than 5.11 pg/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

D. CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant human CRP produced at R&D Systems®.

E. LINEARITY

To assess the linearity of the assay, different samples containing or spiked with high concentrations of CRP and diluted with Reagent Diluent (1×) to produce samples with values within the dynamic range of the assay.

Dilution	Average % of Expected	Range (%)
1:2	98.2	86.9-104.8
1:4	95.3	79.9-106.9
1:8	95.9	83.6-105.5
1:16	84.8	76.5-92.9

F. SAMPLE VALUES

Five serum samples were evaluated for the presence of CRP in this assay. All samples measured ranged from 8156 to 13436 ng/mL with an average of 9710 ng/mL.

G. SPECIFICITY

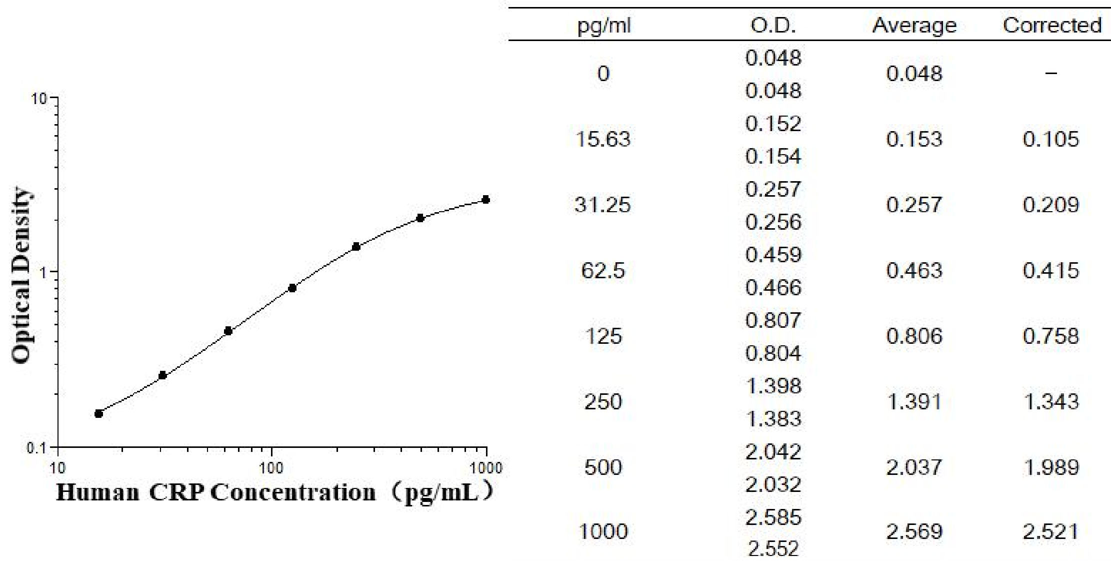
This assay recognizes both natural and recombinant human CRP. The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference.

Recombinant human:	**Other recombinants:**
Pentraxin 2	Mouse CRP
Pentraxin 3	Porcine CRP
	Rat CRP

IV. EXPERIMENT

EXAMPLE STANDARD

The standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



pg/ml	O.D.	Average	Corrected
0	0.048 0.048	0.048	–
15.63	0.152 0.154	0.153	0.105
31.25	0.257 0.256	0.257	0.209
62.5	0.459 0.466	0.463	0.415
125	0.807 0.804	0.806	0.758
250	1.398 1.383	1.391	1.343
500	2.042 2.032	2.037	1.989
1000	2.585 2.552	2.569	2.521

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Human CRP Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse antibody against human CRP.	1 plate
Human CRP Standard	Recombinant human CRP in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume.	2 vials
Human CRP Detection Antibody	Biotinylated CRP polyclonal antibody, lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Streptavidin-HRP A (200×)	200× Streptavidin conjugated to horseradish peroxidase.	1 vial
Reagent Diluent Concentrate (10×)	A 10× concentrated buffered protein base used to dilute standard, samples, detection antibody and HRP.	1 vial
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant with preservatives.	1 vial
TMB Substrate	TMB ELISA Substrate Solution	1 vial
Stop Solution	2 N sulfuric acid.	1 vial
Plate Sealers	Adhesive strips.	3 strips

B. STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2-8 °C. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="9">Opened/
Reconstituted
Reagents</td><td>Streptavidin-HRP A</td><td rowspan="4">May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Diluted Wash Solution</td></tr>
<tr><td>TMB Substrate</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Standard</td><td>Prepare fresh for each assay.</td></tr>
<tr><td>Detection Antibody</td><td>Aliquot and store for up to 1 month at -20 °C in a manual defrost freezer. *</td></tr>
<tr><td>Reagent Diluent Concentrate (10×)</td><td>May be stored for up to 1 month at 2-8 °C.*
Use and discard diluted Reagent Diluent (1×). Prepare fresh for each assay.</td></tr>
<tr><td>Microplate Wells</td><td>Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8 °C.*</td></tr>
</table>

* Provided this is within the expiration date of the kit.

C. OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Test tubes for dilution of standards.
- 100mL and 500 mL graduated cylinder.

D. PRECAUTION

- Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.
- The Stop Solution provided with this kit is an acid solution.
- Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

VI. PREPARATION

A. SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Reagent Diluent (1×).

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Reagent Diluent (1×).

B. SAMPLE PREPARATION

Serum samples recommend a 40,000-fold dilution. For example, add 10 μL of serum into a tube with 1990 μL Reagent Diluent (1×) to prepare a 200-fold diluted sample. Mix through and then pipette 10 μL of prepared 200-fold diluted sample into a tube with 1990 μL Reagent Diluent (1×) to prepare a final 40,000 fold diluted sample. Optimal dilutions should be determined by the end user.

C. REAGENT PREPARATION

Note: *Bring all reagents to room temperature before use.*

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500 mL of Wash Buffer.

Reagent Diluent (1×) - Use deionized or distilled water to prepare Reagent Diluent (1×).

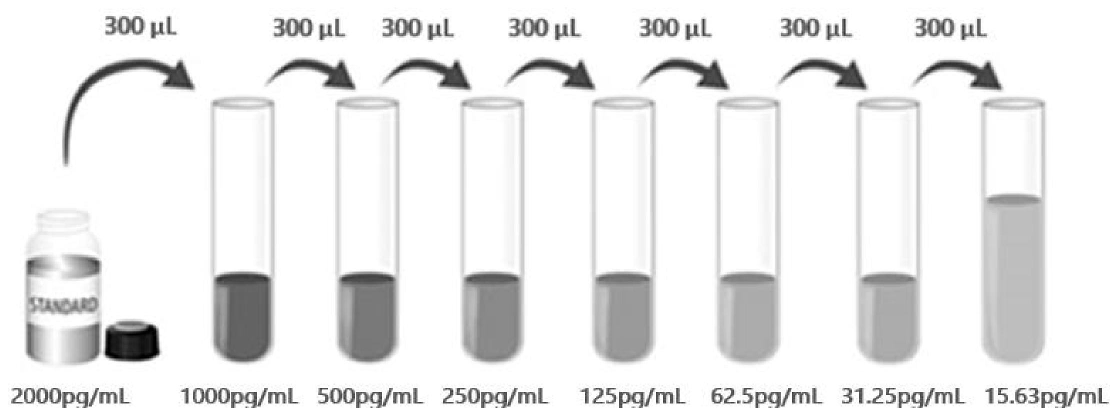
Detection Antibody (1×) - Centrifuge briefly before opening. Reconstitution Volume refer to vial label to prepare Detection Antibody (100×). Allow the Detection Antibody to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Aliquot and store if needed. Dilute to Detection Antibody (1×) with Reagent Diluent (1×). Prepare at least 15 minutes prior to use.

Streptavidin-HRP A (1×) - Centrifuge briefly before opening. Dilute to the working concentration specified on the vial label using Reagent Diluent (1×).

Human CRP Standard - Centrifuge briefly before opening. Refer to the vial label for the reconstitution volume* using Reagent Diluent (1×). This reconstitution produces a stock solution of 2000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

*If you have any question, please seek help from our Technical Support.

Pipette 300 µL of Reagent Diluent (1×) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 1000 pg/mL standard serves as the high standard. The **Reagent Diluent (1×)** serves as the zero standard (0 pg/mL).



D. TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- 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.
- It is recommended that the samples be pipetted within 15 minutes.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- TMB Substrate should remain colorless until added to the plate. Keep Substrate Solution protected from light. TMB Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the TMB substrate. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the TMB substrate.

VII.ASSAY PROCEDURE

Note: *Bring all reagents and samples to room temperature before use. It is recommended that all samples and standards be assayed in duplicate.*

1. Prepare all reagents and working standards 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, and reseal.
3. Add 100 µL of Standard, or prepared sample per well. Cover with the adhesive strip provided. **Incubate for 2 hours at room temperature.** A plate layout is provided for a record of standards and samples assayed. (Serum samples may require dilution. See Sample Preparation section.)
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Solution (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100 µL of the Detection Antibody (1×) diluted in Reagent Diluent, to each well. Cover with a new adhesive strip and **incubate 2 hours at room temperature**.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of the working dilution of Streptavidin-HRP A to each well. Cover the plate and **incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of TMB Substrate to each well. **Incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.**
10. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well within 10 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical

imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

12. CALCULATION OF RESULTS

Average the duplicate readings for each standardand sample and subtract the average zero standard optical density (O.D.). Create a standard curve by reducing the data using computer software capable of generating a four-parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human CRP concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

VIII. REFERENCES

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2. Ahmed, M.S et al. (2012) ISRN Inflamm. 2012:953461.

3. Pepys, M.B. et al. (1979) Nature 278:259.

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PLATE LAYOUT

Use this plate layout to record standards and samples assayed.

产品信息及操作手册

人 C 反应蛋白（CRP）Valukine™ ELISA 试剂盒

目录号：VAL120

适用于定量检测天然和重组人 C 反应蛋白（CRP）的浓度

科研专用，不可用于临床诊断

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有效期详见试剂盒包装标签

Novus 试剂盒确保在你收货日期 3 个月内有效

版本号 202308.3

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I. 背景

C 反应蛋白(CRP)，也被称为 Pentraxin 1，是 Pentraxin 家族中的一种非糖基化蛋白，此家族还包括 Pentraxin 2/SAP 和 Pentraxin 3/TSG-14。CRP 是天然免疫应答的传感器和激活因子(1)。是人体中一种主要的急性期蛋白，在炎症开始时其循环浓度显著升高(2)。而小鼠血清中 CRP 水平在炎症过程中仅略有升高，类似的急性期作用由 Pentraxin 2 填补(3)。CRP 结合、调理并诱导吞噬细菌和凋亡细胞 (4, 6-8)。它通过结合补体级联中的几个蛋白以及巨噬细胞和树突状细胞上的 Fc - RI、Fc - RIIA 和 Fc - RIIB 来调控经典补体通路的激活(6, 7, 9-11, 12-14)。它还促进树突状细胞的成熟和体液免疫(8)。在心血管疾病中，CRP 与氧化 LDL 结合，加重心肌梗死组织损伤，抑制受损血管内皮的修复(5, 15, 16)。

II. 概述

A. 检测原理

本实验采用双抗体夹心 ELISA 法。抗人 CRP 捕获抗体包被于微孔板上，经过孵育，样品和标准品中的 CRP 会与固定在板上的抗体结合，游离的成分被洗去；接着加入生物素化的抗人 CRP 检测抗体进行孵育，洗涤去除未结合的物质后，加入链霉亲和素标记的辣根过氧化物酶(Streptavidin-HRP)孵育。洗涤去除未结合的试剂后加入 TMB 底物溶液（显色剂）。溶液颜色与结合的目标蛋白成正比；加入终止液；用酶标仪测定吸光度。

B. 检测局限

- **仅供科研使用，不可用于体外诊断；**
- 该试剂盒适用于细胞培养上清样本和人血清样本；
- 请在试剂盒有效期内使用；
- 不同试剂盒及不同批号试剂盒的组分不能混用；
- 样本值若大于标准曲线的最高值，应将样本用试剂稀释液（1×）稀释后重新检测；
- 检测结果的不同可由多种因素引起，包括实验人员的操作、移液器的使用方式、洗板技术、反应时间或温度、试剂盒的效期等。

III. 优势

A. 精确度

板内精确度（同一板内不同孔间的精确度）

已知浓度的三个样本，在同一板内分别检测 20 次，以确定板内精确度。

板间精确度（不同板之间的精确度）

已知浓度的三个样本，在不同板中分别检测 20 次，以确定板间精确度。

	板内精确度			**板间精确度**		
样本	1	2	3	1	2	3
平均值（pg/mL）	52.3	207.5	854.4	51.7	204.3	874.0
标准差	1.3	9.6	46.7	1.5	8.0	55.2
CV%	2.6	4.6	5.5	3.0	3.9	6.3

B. 回收率

在细胞培养基样本中掺入检测范围内不同水平的人 CRP，测定其回收率。回收率范围在 84.1-87.8%，平均回收率在 86.9%。

C. 灵敏度

人 CRP 的最低可测剂量（MDD）一般小于 5.11 pg/mL。

MDD 是根据 20 个重复的零标准品孔的吸光度值的平均值加两倍标准差计算得到的相对应浓度。

D. 校正

此 ELISA 试剂盒经由 R&D Systems®生产的 NS0 表达的高纯度重组人 CRP 蛋白所校正。

E. 线性

不同的样本中含有或掺入高浓度的人 CRP，然后用试剂稀释液（1×）将样本稀释到检测范围内，测定其线性。

稀释倍数	平均期待值（%）	范围（%）
1:2	98.2	86.9-104.8
1:4	95.3	79.9-106.9
1:8	95.9	83.6-105.5
1:16	84.8	76.5-92.9

F. 样本值

使用本试剂盒检测了 5 份人血清样本中 CRP 的水平。5 份样本的检测值在 8156-13436 ng/mL 之间，平均值为 9710 ng/mL。

G. 特异性

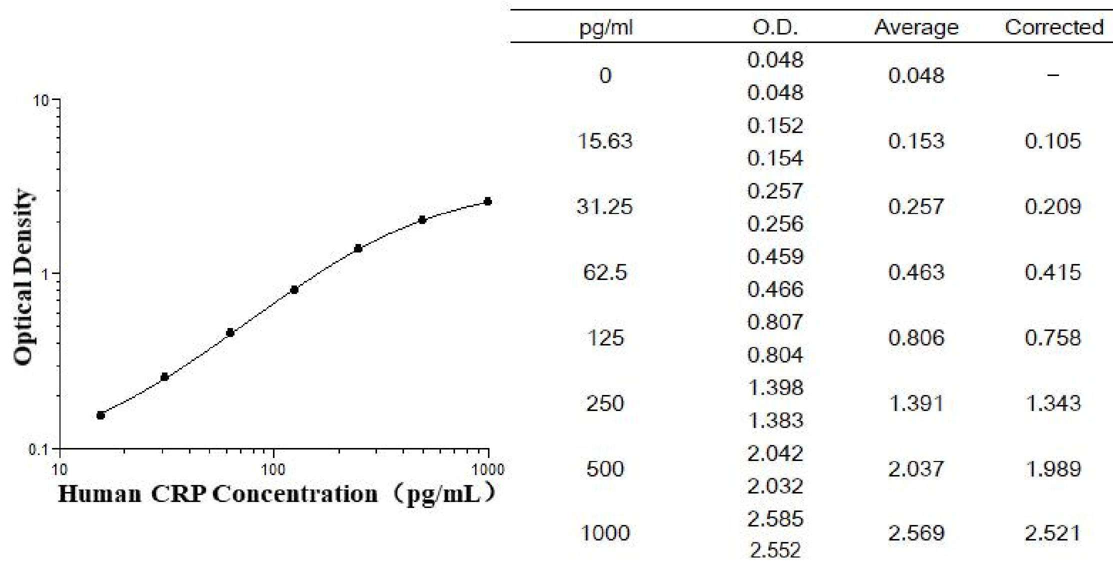
此 ELISA 法可检测天然及重组人 CRP 蛋白。对制备的 50ng/mL 的下列因素进行了测定，无交叉反应或干扰。

Recombinant human：	**Other recombinants：**
Pentraxin 2	Mouse CRP
Pentraxin 3	Porcine CRP
	Rat CRP

IV. 实验

标准曲线实例

该标准曲线数据仅供参考，每次实验应绘制其对应的标准曲线。



pg/ml	O.D.	Average	Corrected
0	0.048	0.048	–
	0.048		
15.63	0.152	0.153	0.105
	0.154		
31.25	0.257	0.257	0.209
	0.256		
62.5	0.459	0.463	0.415
	0.466		
125	0.807	0.806	0.758
	0.804		
250	1.398	1.391	1.343
	1.383		
500	2.042	2.037	1.989
	2.032		
1000	2.585	2.569	2.521
	2.552		

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Human CRP Microplate	包被小鼠抗人 CRP 抗体的 96 孔聚苯乙烯板，8 孔×12 条	1 块板
Human CRP Standard	标准品（冻干粉），参考瓶标签进行重溶	2 瓶
Human CRP Detection Antibody	生物素化的 CRP 检测抗体，冻干粉，参考瓶标签进行重溶	1 瓶
Streptavidin-HRP A（200×）	200×浓缩的链霉亲和素标记的 HRP	1 瓶
Reagent Diluent Concentrate（10×）	浓缩的试剂稀释液（10 ×）用于稀释标准品，样本，检测抗体，HRP	1 瓶
Wash Buffer Concentrate（25×）	浓缩洗涤缓冲液（25 ×）	1 瓶
TMB Substrate	TMB ELISA 底物溶液	1 瓶
Stop Solution	终止液	1 瓶
Plate Sealers	封板膜	3 张

B. 试剂盒储存

<table>
<tr><td>未开封试剂盒</td><td colspan="2">2-8℃储存；请在试剂盒有效期内使用</td></tr>
<tr><td rowspan="8">已打开，稀释或重溶的试剂</td><td>链霉亲和素-HRP A</td><td rowspan="4">2-8℃储存，最多 30 天*</td></tr>
<tr><td>洗涤缓冲液（1×）</td></tr>
<tr><td>TMB 底物溶液</td></tr>
<tr><td>终止液</td></tr>
<tr><td>标准品</td><td>使用时新鲜配制*
标准品-20℃储存，最多 30 天*</td></tr>
<tr><td>检测抗体</td><td>分装，2-8℃储存，最多 30 天*</td></tr>
<tr><td>试剂稀释液（10×）</td><td>2-8℃储存，最多 30 天*
请每次使用新鲜配制的 1×试剂稀释液，多余的丢弃</td></tr>
<tr><td>包被的微孔板条</td><td>将未用的板条放回带有干燥剂的铝箔袋内，密封：2-8℃储存，最多 30 天*</td></tr>
</table>

*必须在试剂盒有效期内

C. 实验所需自备试验器材

- 酶标仪（可测量 450 nm 检测波长的吸收值及 540 nm 或 570 nm 校正波长的吸收值）
- 高精度加液器及一次性吸头
- 蒸馏水或去离子水
- 洗瓶（喷瓶）、多通道洗板器或自动洗板机
- 用来稀释标准品的试管
- 100 mL 及 500 mL 量筒

D. 注意事项

- 试剂盒中的终止液是酸性溶液，使用时请做好眼睛、手、面部及衣服的防护。
- 试剂盒中的一些组分，可能引起皮肤、眼睛和呼吸道刺激或皮肤过敏反应。避免吸入。
- 实验穿戴防护衣服、手套、眼镜和脸的保护罩。使用后请彻底洗手。

VI. 实验前准备

A.　样品收集及储存

细胞培养上清液：颗粒物应离心去除；立刻检测样本。样本收集后若不及时检测，需按一次使用量分装，冻存于-20℃冰箱内，避免反复冻融。样本可能需要用试剂稀释液（1×）稀释。

血清样本: 用血清分离管(SST)分离血清。使血样室温凝集30分钟，然后1000 x g离心15分钟。吸取血清样本之后即刻用于检测，或者分装，-20℃贮存备用。避免反复冻融。样本可能需要用试剂稀释液（1×）稀释。

B.　样本准备工作

血清样本推荐用试剂稀释液（1×）40000倍稀释后进行检测，例如：10 μL血清加到1990 μL试剂稀释液（1×）中，充分混匀，即200倍稀释。然后取10 μL 200倍稀释后的样本加到1990 μL试剂稀释液（1×）中，充分混匀，即制备成40000倍稀释的样本。最佳稀释度应由最终用户确定。

C.　检测前准备工作

使用前请将所有试剂放置于室温。

洗涤液：从冰箱中取出的浓缩洗涤液可能有结晶，属于正常现象；放置室温，轻摇混匀，待结晶完全溶解后再配制洗涤液。可将 20 mL 浓缩洗涤液用蒸馏水或去离子水稀释配置成 500 mL 工作浓度的洗涤液。可 4℃存储。

试剂稀释液（1×）：使用蒸馏水或去离子水稀释配制成试剂稀释液 (1×)。

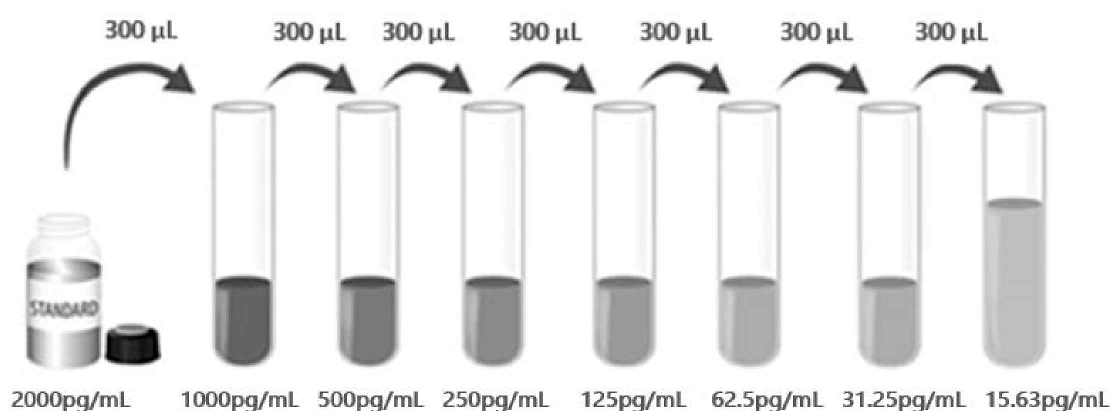
检测抗体（1×）：开盖前请瞬时离心。参考检测抗体瓶标签重溶冻干粉，制备检测抗体（100×）。轻轻震摇至少 15 分钟，其充分溶解。如有需要分装保存。用试剂稀释液（1×） 稀释至检测抗体（1×），至少在使用前 15 分钟准备。

链霉亲和素- HRP A（1×）：开盖前请瞬时离心。用试剂稀释液(1×) 将链霉亲和素- HRP A（200×）稀释至工作浓度。

人 CRP 标准品：开盖前请瞬时离心。冻干标准品的重溶请参考瓶身标签。得到浓度为 2000 pg/mL 标准品母液。轻微震摇至少 15 分钟，使其充分溶解。

***如有疑问，请咨询我们的技术支持。**

每个稀释管中加入 300 µL 试剂稀释液（1×）。将标准品母液参照下图做系列稀释，每管须充分混匀后再移液到下一管。1000 pg/mL 管作标准曲线最高点，试剂稀释液（1×）可用作标准品零点（0 pg/mL）。



D. 技术小提示

- 当混合或重溶蛋白液时，尽量避免起沫；
- 为了避免交叉污染，配置不同浓度标准品、上样、加不同试剂都需要更换枪头。另外不同试剂请分别使用不同的移液槽；
- 建议 15 分钟内完成一块板的上样；
- 每次孵育时，正确使用封板膜可保证结果的准确性；
- TMB 底物溶液在上板前应为无色，请避光保存；加入微孔板后，TMB 底物将由无色变成不同深度的蓝色；
- 终止液上板顺序应同 TMB 底物溶液上板顺序一致；加入终止液后，孔内颜色由蓝变黄；若孔内有绿色，则表明孔内液体未混匀，请充分混合。

VII.操作步骤

使用前请将所有试剂和样本放置于室温，建议所有的实验样本和标准品做复孔检测。

1. 按照上一节的说明，准备好所有需要的试剂和标准品；
2. 从已平衡至室温的密封袋中取出微孔板，未用的板条请放回铝箔袋内，重新封口；
3. 分别将不同浓度标准品，实验样本或者质控品加入相应孔中，每孔 100 μL。用封板膜封住反应孔，**室温孵育 2 小时**。说明书提供了一张 96 孔模板图，可用于记录标准品和试验样本的板内位置；（血清样本需要稀释，详情参见样本制备部分。）
4. 将板内液体吸去，使用洗瓶、多通道洗板器或自动洗板机洗板。每孔加洗涤液 400 μL，然后将板内洗涤液吸去。重复操作 3 次，共洗 4 次。每次洗板尽量吸去残留液体会有助于得到好的实验结果。最后一次洗板结束，请将板内所有液体吸干或将板倒置，在吸水纸拍干所有残留液体；
5. 在每个微孔内加入 100 μL 配置好的检测抗体（1×）。用封板膜封住反应孔，**室温孵育 2 小时**；
6. 重复第 4 步洗板操作；
7. 在每个微孔内加入 100 μL 稀释好的链霉亲和素- HRP A 工作液。用封板膜封住反应孔，**室温孵育 20 分钟，注意避光；**
8. 重复第 4 步洗板操作；
9. 在每个微孔内加入 100 μL TMB 底物溶液，**室温孵育 20 分钟，注意避光；**
10. 在每个微孔内加入 50 μL 终止液，请轻拍微孔板，使溶液混合均匀；
11. 加入终止液后 10 分钟内，使用酶标仪测量 450 nm 的吸光度值，设定 540 nm 或 570 nm 作为校正波长。如果波长校正不可用，以 450 nm 的读数减去 540 nm 或 570 nm 的读数。这种减法将校正酶标板上的光学缺陷。没有校正而直接在 450 nm 处进行的读数可能会更高且更不准确；
12. **计算结果：**将每个标准品和样品的复孔吸光值取平均值，然后减去零标准品平均 OD 值（O.D.），使用计算机软件作四参数逻辑（4-PL）曲线拟合创建标准曲线。另一替代方法是，通过绘制 y 轴上每个标准品的平均吸光值与 x 轴上的浓度来构建标准曲线，并通过图上的点绘制最佳拟合曲线。数据可以通过绘制人 CRP 浓度的对数与 O.D.的对数来线性化，并且最佳拟合线可以通过回归分析来确定。该程序将产生足够但不太精确的数据拟合。

   如果样品被稀释，从标准曲线读取的浓度必须乘以稀释倍数。

VIII. 参考文献

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96 孔模板图

请使用 96 孔模板图来记录标准品及样本在板内的位置