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Human C-Reactive Protein (CRP) ELISA Kit

Catalog No: CS428 **Lot:** **Size:** 1 plate **Expiration:**

Specificity:	Human C-reactive Protein (CRP), native and recombinant
Sensitivity:	< 34 pg/ml
Range:	34 pg/ml to 25 ng/ml
Sample Type:	Cell supernatants, serum, plasma samples.
Storage/Stability	Store unused, at -20°C for up to 1 year or 6 months at 2-8°C from date of receipt.

I: Introduction:

The Cell Sciences® Human CRP (C Reactive Protein) ELISA (Enzyme-Linked Immunosorbent Assay) kit is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of human CRP in serum, plasma, cell culture supernatants and urine. This assay employs an antibody specific for human CRP coated on a 96-well plate. Standards and samples are pipetted into the wells and CRP present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human CRP antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of CRP bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

II: Reagents and materials supplied in the kit:

Items	Quantity	Storage/Stability After Preparation
CS428-A Microplate coated with Anti-Human CRP: 12 strips x 8 wells	96 wells	1 month at 2-8°C
CS428-B Wash Buffer Concentrate (20x)	25 mL	1 month at 2-8°C
CS428-C Human CRP Standard, 1 vial is enough to run each standard in duplicate.	2 vials	1 week at -80°C
CS428-D Assay Diluent D (5x): Standard/Sample - Serum or Plasma	2 x 15 mL	1 month at 2-8°C
CS428-E Assay Diluent B (5x): Standard/Sample/Cell Culture Medium	15 mL	1 month at 2-8°C
CS428-F Detection Antibody: Biotinylated Anti-Human CRP Each vial is enough to coat half the microplate.	2 vials	5 days at 2-8°C
CS428-G Streptavidin-HRP Concentrate (300x)	200 µl	Do not reuse when diluted.
CS428-H TMB One-Step Substrate Reagent (3, 3', 5, 5'-tetramethylbenzidine in buffered solution)	12 mL	n/a
CS428-I Stop Solution (0.2 M Sulfuric Acid)	8 mL	n/a

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III: Storage:

The entire kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C. For prepared reagent storage, see table below.

IV: Additional material required:

1. Microplate reader capable of measuring absorbance at 450 nm
2. Precision pipettes to deliver 2 µl to 1 ml volumes
3. Adjustable 1-25 ml pipettes for reagent preparation
4. 100 ml and 1 liter graduated cylinders
5. Absorbent paper
6. Distilled or deionized water
7. Log-log graph paper or computer and software for ELISA data analysis
8. Tubes to prepare standard or sample dilutions

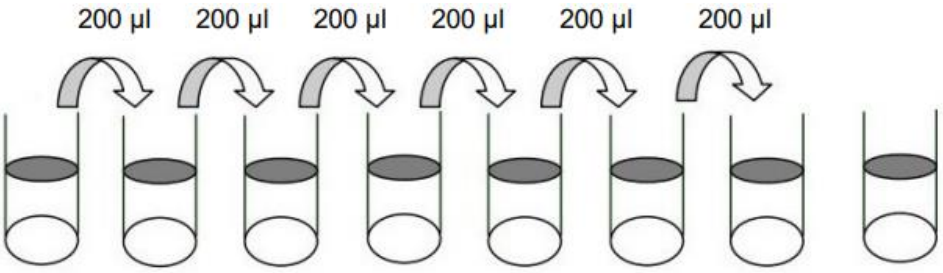
V. Reagent preparation

1. Bring all reagents and samples to room temperature (18 - 25ºC) before use.

2. Assay Diluent D and Assay Diluent B should be diluted 5-fold with deionized or distilled water before use.

3. Sample dilution: 1X Assay Diluent D should be used for dilution of serum, plasma, and cell culture supernatant samples. The suggested dilution for normal serum/plasma is 20,000-fold. For example, add 2 µl of serum/plasma into a tube with 398.0 µl 1X Assay Diluent D to prepare a 200-fold diluted sample. Mix thoroughly and then pipette 3 µl of prepared 200-fold diluted sample into a tube with 297 µl 1X Assay Diluent D to prepare a final 20,000-fold diluted sample. **Note:** Levels of CRP may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.

4. Preparation of standard: Briefly spin a vial of Item C. Add 1,200 µl 1X Assay Diluent D (Item K) into Item C vial to prepare a 25,000 pg/ml standard solution. Dissolve the powder thoroughly by a gentle mix. Pipette 400 µl 1X Assay Diluent D into each tube. Use the stock standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. 1X Assay Diluent D serves as the zero standard (0 pg/ml).



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Zero Standard
Diluent volume	Item C + 1,200 µl	400 µl	400 µl	400 µl	400 µl	400 µl	400 µl	400 µl
Conc.	25000 pg/ml	8333 pg/ml	2778 pg/ml	925.9 pg/ml	308.6 pg/ml	102.9 pg/ml	34.29 pg/ml	0 pg/ml

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5. If the Wash Concentrate (20X) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1X Wash Buffer.

6. Briefly spin the Detection Antibody vial before use. Add 100 µl of 1X Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4ºC for 5 days). The detection antibody concentrate should be diluted 80-fold with 1X Assay Diluent B and used in step 5 of Part VI Assay Procedure.

7. Briefly spin the HRP-Streptavidin concentrate vial and pipette up and down to mix gently before use, as precipitates may form during storage. HRP-Streptavidin concentrate should be diluted 300-fold with 1X Assay Diluent B.

   For example: Briefly spin the vial and pipette up and down to mix gently. Add 40 µl of HRP-Streptavidin concentrate into a tube with 12 ml 1 x Assay Diluent B to prepare a final 300-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

VI. Assay procedure

1. Bring all reagents and samples to room temperature (18 - 25ºC) before use. It is recommended that all standards and samples be run at least in duplicate.

2. Label removable 8-well strips as appropriate for your experiment.

3. Add 100 µl of each standard (see Reagent Preparation step 3) and sample into appropriate wells. Cover wells and incubate for 2.5 hours at room temperature with gentle shaking.

4. Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (300 µl) using a multi-channel Pipette 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 1X prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.

6. Discard the solution. Repeat the wash as in step 4.

7. Add 100 µl of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking.

8. Discard the solution. Repeat the wash as in step 4.

9. Add 100 µl of TMB One-Step Substrate Reagent to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.

VII. Assay procedure summary

1. Prepare all reagents, samples and standards as instructed.

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2. Add 100 µl standard or sample to each well.
   Incubate 2.5 hours at room temperature or over night at 4°C.

⇩

3. Add 100 µl prepared biotin antibody to each well.
   Incubate 1 hour at room temperature.

⇩

4. Add 100 µl prepared Streptavidin solution.
   Incubate 45 minutes at room temperature.

⇩

5. Add 100 µl TMB One-Step Substrate Reagent to each well.
   Incubate 30 minutes at room temperature.

⇩

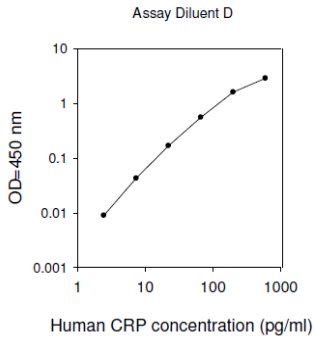
6. Add 50 µl Stop Solution to each well.
   Read at 450 nm immediately.

VIII. Calculation of results

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

A. Typical data

These standard curves are for demonstration only. A standard curve must be run with each assay.



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B. Sensitivity

The minimum detectable dose of CRP is typically less than34 pg/ml.

C. Recovery

Recovery was determined by spiking CRP into normal human serum, plasma and cell culture media. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Serum	116.7	106-127
Plasma	102.2	93-112
Cell culture media	106.2	95-116

D. Linearity

Sample Type		Serum	Plasma	Cell culture media
1:2	Average % of Expected	107.7	113.1	84.76
	Range (%)	95-106	105-123	74-92
1:4	Average % of Expected	77.78	76.18	75.43
	Range (%)	70-88	70-87	68-87

E. Reproducibility

Intra-Assay: CV<10%

Inter-Assay: CV<12%

IX. Specificity

Cross Reactivity: This ELISA kit shows no cross-reactivity with the following cytokines tested: human Angiogenin, BDNF, BLC, ENA-78, FGF-4, IL-1α, IL-1ß, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12 p70, IL-12 p40, IL-13, IL-15, IL-309, IP-10, GM-CSF, Leptin (OB), MCP-1, MCP-3, MDC, MIP-1α, MIP-1ß, MIP-1δ, MMP-1, -2, -3, -10, PARC, RANTES, SCF, TARC, TGF-ß, TIMP-1, TIMP-2, TNF-α, TNF-ß, TPO, VEGF

X. Troubleshooting Guide

Problem	Cause	Solution
1. Poor standard curve	1. Inaccurate pipetting	1. Check pipettes
	2. Improper standard dilution	2. Ensure a brief spin of Item C and dissolve the powder thoroughly by a gentle mix.
2. Low signal	1.Too brief incubation times	1. Ensure sufficient incubation time; assay procedure step 2 may change to over night
	2. Inadequate reagent volumes or improper dilution	2. Check pipettes and ensure correct preparation
3. Large CV	1. Inaccurate pipetting	1. Check pipettes
4. High background	1. Plate is insufficiently washed	1. Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	2. Contaminated wash buffer	2. Make fresh wash buffer
5. Low sensitivity	1. Improper storage of the ELISA kit	1. Store your standard at<-20°C after reconstitution, others at 4 °C. Keep substrate solution protected from light
	2. Stop solution	2. Stop solution should be added to each well before measure