

For the quantitation of human CRP concentrations in cell culture supernatants, serum, and plasma.

### INTRODUCTION

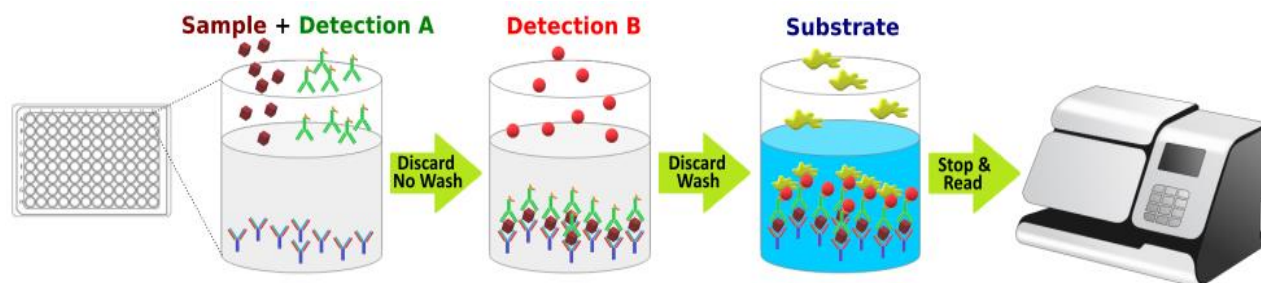
C-Reactive Protein (CRP) is a non-glycosylated protein and a member of the pentameric family of proteins characterized by a cyclic pentameric structure. In mature humans, CRP has 206 amino acids, which are linked by non-covalent bonds to form a pentamer. The CRP acts as a sensor and activator of the innate immune response in humans. Also, when inflammation occurs, circulating concentrations of CRP increase dramatically, suggesting that it is a major acute phase protein. Studies have shown that high levels of CRP in humans are associated with an increased risk of cardiovascular disease, and that it exacerbates tissue damage in coronary infarcts and inhibits repair of damaged vascular endothelium.

The Fast Human CRP ELISA is a solid phase ELISA designed to measure human CRP levels in cell culture supernatants, serum, and plasma. The main feature is that **the kit uses our novel proprietary approaches to combine samples and detections into a one-step instead of the complicated traditional methods. It makes the assay simple, easy, accurate and fast. The measurement can be finished in 1 hour, not needing 4-5 hours (Fig. 1).** The detection range is from 7 to 500 pg/mL. The levels of human CRP samples are parallel to the standard curves obtained using the kit standards linearly. These results indicate that this kit can be used to determine relative mass values for natural human CRP protein.

### PRINCIPLE OF THE ASSAY

This assay employs our novel proprietary sandwich enzyme immunoassay techniques (See Fig. 1). A monoclonal antibody specific for human CRP was pre-coated onto a microplate. Standards or samples and Detection Antibody are pipetted into the wells, and concurrently incubated for 45min. Then, aspirate each well, no wash, directly add Streptavidin-HRP, incubate the complex. Following a wash to remove any unbound antibody and samples, an ultra-sensitive TMB substrate solution is added to the wells for color development. The color intensity is in proportion to the amount of CRP bound in the initial step. The intensity of the color is measured by plate read at 450 nm.

**Fig. 1: Assay Principle:**



### KIT CONTENT AND STORAGE CONDITIONS

PART	PART#	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED
Human CRP Microplate	TBS3209A	96 well microplate (12 strips of 8 wells) coated with a Capture Antibody specific for human CRP.	The unused wells can be stored the sealed foil pouch containing the desiccant pack for up to 1 month at 2-8 °C.
Human CRP Standard	TBS3209B	30ul of Recombinant human CRP protein (25 ng/mL).	Aliquot and store at -20 °C for up to 1 month in a manual defrost freezer. Avoid repeated freeze-thaw cycles.
Detection A	TBS3209C	2.1 ml of human CRP antibody.	May be stored for up to 3 months at 2-8 °C.*
Detection B	TBS3209D	12 ml of Streptavidin-HRP	
Assay Diluent	TBS3209E	12 ml of a buffered protein base with preservatives.	
10x Wash Buffer	TBS3000W	12 ml of concentrated solution (10x).	
TMB Substrate	TBS3000T	12 ml of ultra-sensitive TMB substrate.	
Stop Solution	TBS3000S	6 ml of 2 N sulfuric acid.	

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

The kit contains sufficient materials to run an ELISA on one 96 well plate.

**PRECAUTIONS**

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

**REAGENT PREPARATION**

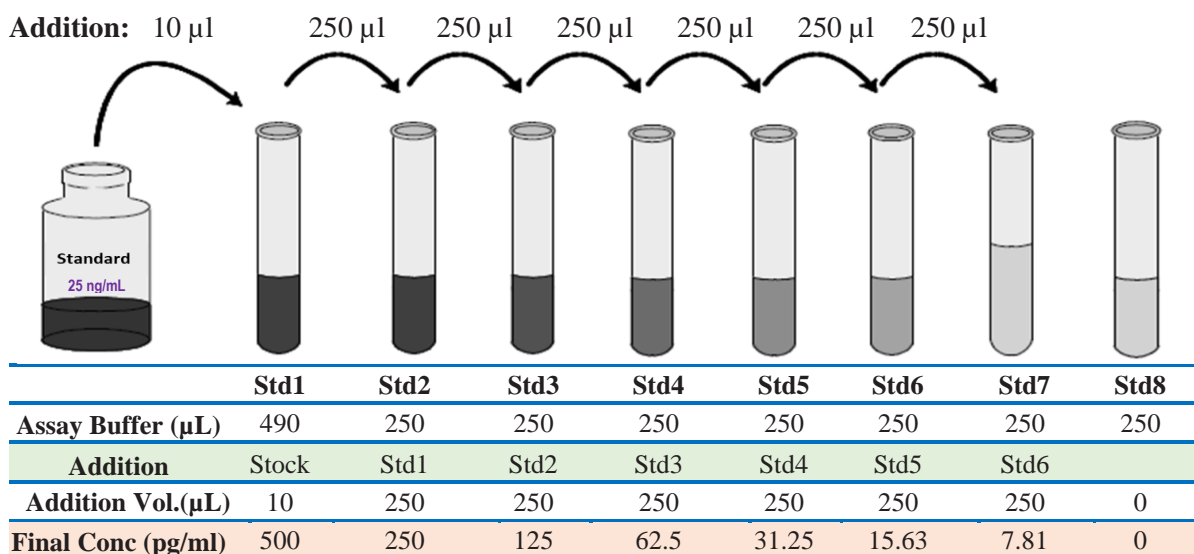
**Bring all reagents to room temperature before use.**

**Wash Buffer:** Add 10 mL of Wash Buffer Concentrate (10x) to 90 mL of deionized distilled water to prepare 100 mL of Wash Buffer (*If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved.*).

**Human CRP Standard Preparation:**

1. Label test tubes as #1 through #8. Pipet 490  $\mu\text{L}$  of 1x Assay Diluent into tube #1, and 250  $\mu\text{L}$  into tubes #2 to #8 as diagram below (Fig. 2).
2. Add 10  $\mu\text{L}$  of the Human CRP Standard stock solution (25 ng/mL) by dilution of 50 times to tube #1(500pg/mL) and mix.
3. Make 2 x serial dilutions of the standard using the 500pg/mL standard solution in tube#1 from tube #2 through #7 with sequential transfer of 250  $\mu\text{L}$  to the next concentration. Mix each tube thoroughly before the next transfer. The standard concentration in tube 1 through 7 will be 500; 250; 125; 62.5; 31.25; 15.63 and 7.81 pg/mL. Tube# 8 is Standard 0.

**Fig. 2 Diagram for human CRP standard preparation**



**ASSAY PROCEDURE**

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

1. Add 80  $\mu\text{L}$  of standard, sample, or control per well.
2. Add 20  $\mu\text{L}$  of **Detection A** to the above standard and sample of each well, thoroughly mix. Cover with the adhesive sealer. Incubate at **RT for 45 min.**
3. Aspirate each well (**no wash**). Invert the plate and blot it against clean paper towels.
4. Add 100  $\mu\text{L}$  of **Detection B** to each well. Incubate at **RT for 30min.**
5. Aspirate each well, and wash for 3 times by filling each well with 200  $\mu\text{L}$  Wash Buffer (*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.
6. Add 100  $\mu\text{L}$  of **TMB Substrate** to each well. Incubate **at RT for 10-20min** (*Protect from light*). The color becomes blue. If the color is light, the incubation time can be longer.

7. Add 50 µL of **Stop Solution** to each well. The color in the well should change from blue to yellow (gently tap the plate to ensure thorough mixing).
8. Determine the optical density of each well within 5 minutes, using a microplate reader at 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.

**CALCULATION OF RESULTS**

Average the duplicate readings for each standard, control, and sample subtract the average zero standard optical density (O.D.).

Create a standard curve 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.

**TYPICAL DATA**

This standard curve ( $R^2=0.9985$ ) is provided for demonstration only. A standard curve should be generated for each set of samples assayed. Fig. 3 is an example of typical Data.

**SENSITIVITY**

The minimum detectable dose (MOD) of human CRP is typically 3.0 pg/ml.

The Intra-assay CV is 4.9% the Inter-assay CV is 8.1%.

**SPECIFICITY**

This assay recognizes natural and recombinant human CRP.

**RELATIVE PRODUCTS**

- Human IL-1IL-1β ELISA (TBS3219)
- Human IL-2 ELISA (TBS3220)
- Human IL-4 ELISA (TBS3221)
- Human IL-6 ELISA (TBS3223)
- Human IL-7 ELISA (TBS3224)
- Human IL-8 ELISA (TBS3225)
- Human IL-10 ELISA (TBS3226)
- Human IL-13 ELISA (TBS3227)
- Human IL-17 ELISA (TBS3228)
- Human IL-22 ELISA (TBS3229)
- Human IFN-gamma ELISA (TBS3230)
- Human TGF- β1 ELISA (TBS3232)
- Human GM-CSF ELISA (TBS3233)
- Human MIP-1α ELISA (TBS3234)
- Human TNF-α ELISA (TBS3235)

**For research use only.**

