Human CRP ELISA Kit

CAT#: orb180677 [ELISA MANUAL]

Assay Principle

The Biorbyt Human CRP ELISA (Enzyme-Linked Immunosorbent Assay) kit is a solid phase immunoassay specially designed to measure Human CRP with a 96-well strip plate that is pre-coated with antibody specific for CRP. The detection antibody is a biotinylated antibody specific for CRP. The capture antibody is monoclonal antibody from mouse, the detection antibody is polyclonal antibody from goat. The kit contains recombinant Human CRP with immunogen: Expression system for standard: NSO; Immunogen sequence: F17-P224. The kit is analytically validated with ready to use reagents.

To measure Human CRP, add standards and samples to the wells, then add the biotinylated detection antibody. Wash the wells with PBS or TBS buffer, and add Avidin-Biotin-Peroxidase Complex (ABC-HRP). Wash away the unbounded ABC-HRP with PBS or TBS buffer and add TMB. TMB is substrate to HRP and will be catalyzed to produce a blue color product, which changes into yellow after adding acidic stop solution. The density of the yellow product is linearly propotional to Human CRP in the sample. Read the density of the yellow product in each well using a plate reader, and benchmark the sample wells' readings against the standard curve to determine the concentration of Human CRP in the sample.

Overview

Product Name	Human CRP ELISA Kit
Reactive Species	Human
Size	96 well
Description	Sandwich High Sensitivity ELISA kit for Quantitative Detection of Human CRP. 96wells/kit, with removable strips.
Sensitivity	<10pg/ml

*The sensitivity or the minimum detectable dose (MDD) is the lower limit of target protein that can be detected by the kit. It is determined by adding two standard deviations to the mean O.D. value of twenty (20) blank wells and calculating the corresponding concentration.

Detection Range 312pg/ml-20000pg/ml

Storage Instructions Store at 4°C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw cycles(Shipped with wet ice.)

Uniprot ID P02741

Technical Details

Capture/Detection Antibodies The capture antibody is monoclonal antibody from mouse, the detection antibody is polyclonal antibody from goat.

Specificity Natural and recombinant Human CRP

Immunogen Expression system for standard: NSO; Immunogen sequence: F17-P224

Cross Reactivity There is no detectable cross-reactivity with other relevant proteins.

Kit Components/Materials Provided

Description	Quantity	Volume
Anti-Human CRP Pre-coated 96-well strip microplate	1	12 strips of 8 wells
Human CRP Standard	2	20ng/tube
Human CRP Biotinylated antibody (100x)	1	130 µl
Avidin-Biotin-Peroxidase Complex (100x)	1	130 µl
Sample Diluent	1	30ml
Antibody Diluent	1	12ml

Avidin-Biotin-Peroxidase Diluent	1	12ml
Color Developing Reagent (TMB)	1	10ml
Stop Solution	1	10ml
Plate Sealers	4	Piece

Required Materials That Are Not Supplied

Microplate Reader capable of reading absorbance at 450nm.

Automated plate washer (optional)

Pipettes and pipette tips capable of precisely dispensing 0.5 µl through 1 ml volumes of aqueous solutions.

Multichannel pipettes are recommended for large amount of samples.

Deionized or distilled water.

500ml graduated cylinders.

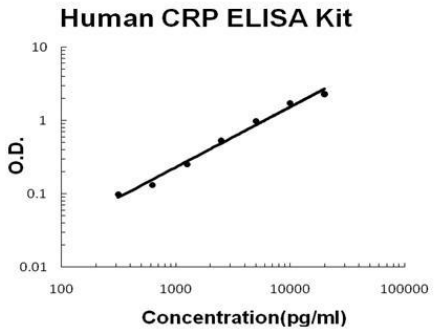
Test tubes for dilution.

Human CRP Reactive Protein ELISA Kit Standard Curve Example

Highest O.D. value might be higher or lower than in the example. The experiment result is statistically significant if the highest O.D. value is no less than 1.0.

Concentration(pg/ml)	***0***	***312***	***625***	***1250***	***2500***	***5000***	***10000***	***20000***
O.D.	***0.043***	***0.099***	***0.131***	***0.253***	***0.530***	***0.978***	***1.730***	***2.288***

Human CRP ELISA Kit standard curve



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

Intra/Inter Assay Variability

Biorbyt spend great efforts in documenting lot to lot variability and make sure our assay kits produce robust data that are reproducible.

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

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

	Intra-Assay Precision			*Inter-Assay Precision*		
Sample	*1*	*2*	*3*	*1*	*2*	*3*
n	*16*	*16*	*16*	*24*	*24*	*24*
Mean(pg/ml)	*593*	*1488*	*8142*	*565*	*1551*	*8792*
Standard deviation	*36.17*	*62.49*	*643.21*	*38.42*	*75.99*	*861.61*
CV(%)	*6.1%*	*4.2%*	*7.9%*	*6.8%*	*4.9%*	*9.8%*

Reproducibility

To assay reproducibility, three samples with differing target protein concentrations were assayed using four different lots.

Lots	*Lot1 (pg/ml)*	*Lot2 (pg/ml)*	*Lot3 (pg/ml)*	*Lot4 (pg/ml)*	*Mean (pg/ml)*	*Standard Deviation*	*CV (%)*
Sample 1	*593*	*514*	*598*	*593*	*574*	*34.98*	*6%*
Sample 2	*1488*	*1753*	*1588*	*1771*	*1650*	*117.62*	*7.1%*
Sample 3	*8142*	*7585*	*8200*	*7898*	*7956*	*242.45*	*3%*

*number of samples for each test n=16

Preparation Before The Experiment

Item	**Preparation**
All reagents	Bring all reagents to 37°C prior to use. The assay can also be done at room temperature however we recommend doing it at 37°C for best consistency with our QC results. Also the TMB incubation time estimate (15-20min) is based on 37°C.
Wash buffer	Dissolve the included powder in 1000ml of deionized water. Excess wash buffer can be stored for up to one week at 4°C.
Biotinylated Anti-Human CRP	It is recommended to prepare this reagent immediately prior to use by diluting the Human CRP Biotinylated antibody (100x) 1:100 with Antibody Diluent. Prepare 100 µl by adding 1 µl of Biotinylated antibody (100x) to 99 µl

	of Antibody Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.
Avidin-Biotin-Peroxidase Complex	It is recommended to prepare this reagent immediately prior to use by diluting the Avidin-Biotin-Peroxidase Complex (100x) 1:100 with Avidin-Biotin-Peroxidase Diluent. Prepare 100 µl by adding 1 µl of Avidin-Biotin-Peroxidase Complex (100x) to 99 µl of Avidin-Biotin-Peroxidase Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.
Human CRP Standard	It is recommended that the standards be prepared no more than 2 hours prior to performing the experiment. Use one 20ng of lyophilized Human CRP standard for each experiment. Gently spin the vial prior to use. Reconstitute the standard to a stock concentration of 20ng/ml using 1ml of sample diluent. Allow the standard to sit for a minimum of 10 minutes with gentle agitation prior to making dilutions.
Microplate	The included microplate is coated with capture antibodies and ready-to-use. It does not require additional washing or blocking. The unused well strips should be sealed and stored in the original packaging.

Dilution of Human CRP Standard

1. Number tubes 1-8. Final Concentrations to be Tube # 1 –20000pg/ml, #2 –10000pg/ml, #3 – 5000pg/ml, #4 – 2500pg/ml, #5 – 1250pg/ml, #6 – 625pg/ml, #7 – 312.5pg/ml, #8 – 0.0 (Blank).
2. For standard #1, add 1000µl of undiluted standard stock solution to tube #1.
3. Add 300 µl of sample diluent to tubes # 2-7.
4. To generate standard #2, add 300 µl of standard #1 from tube #1 to tube #2 for a final volume of 600 µl. Mix thoroughly.

5. To generate standard #3, add 300 µl of standard #2 from tube #2 to tube #3 for a final volume of 600 µl. Mix thoroughly.

6. Continue the serial dilution for tube #4-7.

7. Tube #8 is a blank standard to be used with every experiment.

Sample Preparation and Storage

These sample collection instructions and storage conditions are intended as a general guideline and the sample stability has not been evaluated.

Sample Type	Procedure
Cell culture supernatants	Clear sample of particulates by centrifugation, assay immediately or store samples at -20°C.
Serum	Use a serum separator tube (SST) and allow serum to clot at room temperature for about four hours. Then, centrifuge for 15 min at approximately 1,000 x g. assay immediately or store samples at -20°C.
Plasma	Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 min at approximately 1,000 x g. Assay immediately or store samples at -20°C. *Note: it is important to not use anticoagulants other than the ones described above to treat plasma for other anticoagulants could block the antibody binding site.
Cell lysates	Lyse the cells, make sure there are no visible cell sediments. Centrifuge cell lysates at approximately 10000 X g for 5 min. Collect the supernatant.

Sample Dilution

The target protein concentration should be estimated and appropriate sample dilutions should be selected such that the final protein concentration lies near the middle of the linear dynamic range of the assay.

It is recommended to prepare 150 µl of sample for each replicate to be assayed. The samples should be diluted with sample diluent and mixed gently.

Assay protocol

It is recommended that all reagents and materials be equilibrated to 37°C/room temperature prior to the experiment (see Preparation Before The Experiment if you have missed this information).

1. Prepare all reagents and working standards as directed previously.
2. Remove excess microplate strips from the plate frame and seal and store them in the original packaging.
3. Add 100 µl of the standard, samples, or control per well. At least two replicates of each standard. sample, or control is recommended.
4. Cover with the plate sealer provided and incubate for 120 minutes at RT (or 90 min. at 37 °C).
5. Remove the cover and discard the liquid in the wells into an appropriate waste receptacle. Invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
6. Add 100 µl of the prepared 1x Biotinylated Anti-Human CRP antibody to each well.
7. Cover with plate sealer and incubate for 90 minutes at RT (or 60 minutes at 37°C).
8. Wash the plate 3 times with the 1x wash buffer.
    a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any

remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.

b. Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).

c. Repeat steps a-b 2 additional times.

9. Add 100 µl of the prepared 1x Avidin-Biotin-Peroxidase Complex into each well and incubate for 40 minutes at RT (or 30 minutes at 37°C).

10. Wash the plate 5 times with the 1x wash buffer.

a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.

b. Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).

c. Repeat steps a-b 4 additional times.

11. Add 90 µl of Color Developing Reagent to each well and incubate in the dark for 30 minutes at RT (or 25-30 minutes at 37°C). (The optimal incubation time must be empirically determined. A guideline to look for is blue shading the top four standard wells, while the remaining standards remain clear.)

12. Add 100 µl of Stop Solution to each well. The color should immediately change to yellow.

13. Within 30 minutes of stopping the reaction, the O.D. absorbance should be read with a microplate reader at 450nm.

Data Analysis

Average the duplicate readings for each standard, sample, and control. Subtract the average zero standard O.D. reading.

It is recommended that a standard curve be created using computer software to generate a four parameter logistic (4-PL) curve-fit. A free program capable of generating a four parameter logistic (4-PL) curve-fit can be found online at: www.myassays.com/four-parameter-logistic-curve.assay.

Alternatively, plot the mean absorbance for each standard against the concentration. The measured concentration in the sample can be interpolated by using linear regression of each average relative OD against the standard curve generated using curve fitting software. This will generate an adequate but less precise fit of the data.

For diluted samples, the concentration reading from the standard curve must be multiplied by the dilution factor.

Background on CRP

C Reactive Protein (CRP) is a major acute phase reactant synthesized primarily in the liver hepatocytes. It is composed of 5 identical, 21,500-molecular weight subunits. CRP mediates activities associated with preimmune nonspecific host resistance. CRP shows the strongest association with cardiovascular events. It is detectable on the surface of about 4% of normal peripheral blood lymphocytes. Acute phase reactant CRP is produced in the liver.