

Version 1 Last updated 29 June 2017

ab222864

Human Complement Factor P ELISA Kit

For the quantitative measurement of human Complement Factor P in serum, plasma, saliva, cerebral spinal fluid, urine and cell culture samples.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

| | |
|-------------------------------------|----|
| 1. Overview | 1 |
| 2. Protocol Summary | 2 |
| 3. Precautions | 3 |
| 4. Storage and Stability | 3 |
| 5. Limitations | 4 |
| 6. Materials Supplied | 4 |
| 7. Materials Required, Not Supplied | 5 |
| 8. Technical Hints | 6 |
| 9. Reagent Preparation | 7 |
| 10. Standard Preparation | 8 |
| 11. Sample Preparation | 9 |
| 12. Assay Procedure | 11 |
| 13. Typical Data | 13 |
| 14. Typical Sample Values | 14 |
| 15. Assay Specificity | 17 |
| 16. Species Reactivity | 17 |
| 17. Troubleshooting | 18 |
| 18. Notes | 21 |

1. Overview

The Human Complement Factor P ELISA (Enzyme-Linked Immunosorbent Assay) Kit (ab222864) is designed for detection of Complement Factor P in human plasma, serum, urine, saliva, cerebral spinal fluid, and cell culture samples.

This assay employs a quantitative sandwich enzyme immunoassay technique that measures human Complement Factor P in approximately 4 hours. A polyclonal antibody specific for human Complement Factor P has been pre-coated onto a 96-well microplate with removable strips. Complement Factor P in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for human Complement Factor P, which is recognized by a streptavidin-peroxidase (SP) conjugate. All unbound material is washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Complement factor P (CFP), also known as properdin, is a plasma glycoprotein that positively regulates the alternative complement pathway convertases (C3bBb) of the innate immune system. CFP consists of 442-amino acids with a molecular weight of 53 kDa and is composed of multiple identical protein subunits. The subunits bind to each other in a head to tail manner to form cyclic dimers, trimers, tetramers, pentamers, and higher cyclic oligomers. It is mainly produced by neutrophils but also by monocytes, T cells, and bone marrow progenitor cells. CFP binds to many microbial surfaces and apoptotic cells. CFP also stabilizes the C3- and C5-convertase enzyme complexes in a feedback loop that ultimately leads to formation of the membrane attack complex and lysis of the target cell.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed



Add 50 μ L standard or sample to appropriate wells.

Incubate for 2 hours



Wash wells. Add 50 μ L Biotinylated Antibody to all wells.

Incubate for 1 hour



Wash wells. Add 50 μ L Streptavidin-Peroxidase Conjugate to all wells.

Incubate for 30 minutes



Wash wells. Add 50 μ L Chromogen Substrate to all wells.

Incubate for 15 minutes



Add 50 μ L Stop Solution and read OD at 450 nm

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at +4°C immediately upon receipt, apart from the Biotinylated Antibody and the Streptavidin-Peroxidase Conjugate, which should be stored at -20°C. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

| Item | Quantity | Storage Condition |
|---|-----------|-------------------|
| Anti-Human Complement Factor P coated Microplate (12 x 8 wells) | 96 wells | +4°C |
| Human Complement Factor P Standard | 1 vial | +4°C |
| Biotinylated Human Complement Factor P | 120 µL | -20°C |
| 10X Diluent M Concentrate | 30 mL | +4°C |
| 20X Wash Buffer Concentrate | 2 x 30 mL | +4°C |
| 100X Streptavidin-Peroxidase Conjugate | 80 µL | -20°C |
| Chromogen Substrate | 8 mL | +4°C |
| Stop Solution | 12 mL | +4°C |
| Sealing Tapes | 3 units | +4°C |

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at 450.
- Deionized water.
- Multi- and single-channel pipettes.
- Tubes for standard dilution.

8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 10X Diluent M Concentrate:

If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Diluent M Concentrate 10-fold with reagent grade water. Store for up to 30 days at +4°C.

9.2 Biotinylated Human Complement Factor P:

Spin down the antibody briefly and dilute the desired amount of the antibody 50-fold with Diluent M. The undiluted antibody should be stored at -20°C.

9.3 20X Wash Buffer Concentrate:

If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 20-fold with reagent grade water.

9.4 100X Streptavidin-Peroxidase Conjugate:

Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 100-fold with Diluent M. The undiluted antibody should be stored at -20°C.

9.5 Anti-Human Complement Factor P coated Microplate (12 x 8 wells):

Ready to use 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human CFP. Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator at +4°C.

9.6 Chromogen Substrate:

Ready to use stabilized peroxidase chromogen substrate tetramethylbenzidine.

9.7 Stop Solution:

Ready to use 0.5 N hydrochloric acid.

9.8 Sealing Tapes:

Ready to use. Each kit contains 3 pre-cut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 Reconstitute the Factor P Stock to generate a 10 ng/mL **Standard #1**.

10.1.1 First consult the Factor P Standard vial to determine the mass of protein in the vial.

10.1.2 Calculate the appropriate volume of 1X Diluent M to add when resuspending the Factor P Standard vial to produce a 10 ng/mL Factor P Standard stock by using the following equation:

C_S = Starting mass of Factor P Standard stock (see vial label) (ng)

C_F = 10 ng/mL Factor P Standard #1 final required concentration

V_D = Required volume of 1X Diluent M for reconstitution (μ L)

Calculate total required volume 1X Diluent M for resuspension:

$$(C_S / C_F) * 1,000 = V_D$$

Example:

NOTE: This example is for demonstration purposes only. Please remember to check your standard vial for the actual amount of standard provided.

C_S = 8 ng of Factor P Standard in vial

C_F = 10 ng/mL Factor P **Standard #1** final concentration

V_D = Required volume of 1X Diluent M for reconstitution

$$(8 \text{ ng} / 10 \text{ ng/mL}) * 1,000 = 800 \mu\text{L}$$

- 10.1.3 First briefly centrifuge the Factor P Standard Vial to collect the contents on the bottom of the tube.
- 10.1.4 Reconstitute the Factor P Standard vial by adding the appropriate calculated amount VD of 1X Diluent M to the vial to generate the 10 ng/mL Factor P **Standard #1**. Mix gently and thoroughly.
- 10.2 Allow the reconstituted 10 ng/mL Factor P **Standard #1** to sit for 10 minutes with gentle agitation prior to making subsequent dilutions
- 10.3 Label seven tubes #2 – 8.
- 10.4 Prepare duplicate or triplicate standard points by serially diluting the standard stock solution (10 ng/mL) 1 in 2 with Diluent M to produce 5, 2.5, 1.25, 0.625, 0.313, and 0.156 ng/ml solutions. Diluent M serves as the zero standard (0 ng/mL).
- 10.5 Add 120 µL of 1X Diluent M to tube #2 – 8.
- 10.6 To prepare **Standard #2**, add 120 µL of the **Standard #1** into tube #2 and mix gently.
- 10.7 To prepare **Standard #3**, add 120 µL of the **Standard #2** into tube #3 and mix gently.
- 10.8 Using the table below as a guide, prepare subsequent serial dilutions.

| Standard # | Volume to dilute (µL) | Volume Diluent M (µL) | Human Factor P (ng/mL) |
|------------|-----------------------|-----------------------|------------------------|
| 1 | Step 10.1 | | 10 |
| 2 | 120 µL Standard #1 | 120 | 5 |
| 3 | 120 µL Standard #2 | 120 | 2.5 |
| 4 | 120 µL Standard #3 | 120 | 1.25 |
| 5 | 120 µL Standard #4 | 120 | 0.625 |
| 6 | 120 µL Standard #5 | 120 | 0.313 |
| 7 | 120 µL Standard #6 | 120 | 0.156 |
| 8 (Blank) | N/A | 120 | 0 |

11. Sample Preparation

11.1 Plasma:

Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x g for 10 minutes and collect plasma. A 40,000-fold sample dilution is suggested into Diluent M; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant).

11.2 Serum:

Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3,000 x g for 10 minutes and remove serum. An 8,000-fold sample dilution is suggested into Diluent M; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

11.3 Cell Culture Supernatants:

Centrifuge cell culture media at 3,000 x g for 10 minutes at 4°C to remove debris and collect supernatants. Dilute samples if necessary. The undiluted samples can be stored at -20°C or below. Avoid repeated freeze-thaw cycles.

11.4 Urine:

Collect urine using sample pot. Centrifuge samples at 800 x g for 10 minutes. The sample is suggested for use at 1X; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

11.5 Cerebral Spinal Fluid:

Collect cerebral spinal fluid (CSF) using sample pot. Centrifuge samples at 3,000 x g for 10 minutes. A 4-fold sample dilution is suggested into Diluent M; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -80°C for up to 3 months. Avoid repeated freeze-thaw cycles.

11.6 Saliva:

Collect saliva using sample tube. Centrifuge samples at 800 x g for 10 minutes. A 4-fold sample dilution is suggested into Diluent M; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
 - We recommend that you assay all standards, controls and samples in duplicate.
- 12.1 Prepare all reagents, working standards, and samples as directed in the previous sections. The assay is performed at room temperature (20-25°C).
 - 12.2 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.
 - 12.3 Add 50 μ L of Complement Factor P Standard or sample per well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
 - 12.4 Wash five times with 200 μ L of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 μ L of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.
 - 12.5 Add 50 μ L of Biotinylated Complement Factor P Antibody to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 1 hour.
 - 12.6 Wash the microplate as described in step 12.4.
 - 12.7 Add 50 μ L of Streptavidin-Peroxidase Conjugate per well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
 - 12.8 Wash the microplate as described in step 12.4.
 - 12.9 Add 50 μ L of Chromogen Substrate per well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Incubate for 15 minutes or till the optimal color density develops.
 - 12.10 Add 50 μ L of Stop Solution to each well. The color will change from blue to yellow. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed.

12.11

12.12 Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

12.13 Analyze the data as described below.

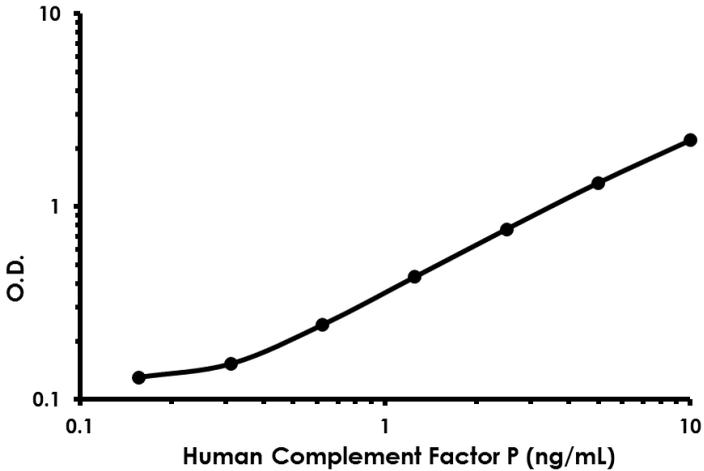
12.1.1 Calculate the mean value of the duplicate or triplicate readings for each standard and sample.

12.1.2 To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance (OD) on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.

12.1.3 Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

13. Typical Data

Typical standard curve – data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.



| Standard Curve Measurements | | | |
|-----------------------------|------------|-------|----------|
| Concentration (ng/mL) | O.D 450 nm | | Mean O.D |
| | 1 | 2 | |
| 10 | 2.303 | 2.299 | 2.301 |
| 5 | 1.426 | 1.421 | 1.425 |
| 2.5 | 0.881 | 0.839 | 0.860 |
| 1.25 | 0.538 | 0.518 | 0.528 |
| 0.625 | 0.344 | 0.338 | 0.341 |
| 0.313 | 0.256 | 0.244 | 0.250 |
| 0.156 | 0.230 | 0.224 | 0.227 |
| 0 | 0.100 | 0.094 | 0.097 |

Figure 1. Example of human Complement Factor P standard curve in 1X Diluent M. The standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

14. Typical Sample Values

SENSITIVITY –

The minimum detectable dose (MDD) of Human Complement Factor P as calculated by 2 standard deviations from the mean of a zero standard was established to be 0.1 ng/ml.

PRECISION –

Intra-assay precision was determined by testing replicates of three plasma samples twenty times in one assay.

Inter-assay precision was determined by testing three plasma samples in twenty assays.

| | Intra-assay Precision | | | Inter-Assay Precision | | |
|----------------|-----------------------|-----|-----|-----------------------|-----|------|
| Sample | 1 | 2 | 3 | 1 | 2 | 3 |
| N | 20 | 20 | 20 | 20 | 20 | 20 |
| CV (%) | 5.1 | 4.8 | 5.0 | 9.9 | 9.7 | 10.2 |
| Average CV (%) | 5.0 | | | 9.9 | | |

RECOVERY –

| | |
|-----------------------------|---------------|
| Standard Added Value | 0.625-5 ng/ml |
| Recovery (%) | 88 – 113 % |
| Average Recovery (%) | 96% |

Linearity of Dilution

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Plasma and serum samples were serially-diluted to test for linearity.

| Average Percentage of Expected Value (%) | |
|---|--------|
| Dilution Factor | Plasma |
| 4000 | 94% |
| 8000 | 101% |
| 16000 | 96% |

15. Assay Specificity

This kit recognizes Human Complement Factor P in serum, plasma, saliva, cerebral spinal fluid, urine and cell culture supernatant samples.

CROSS REACTIVITY –

| Protein | Cross Reactivity (%) |
|----------------|----------------------|
| Complement C1 | 10% |
| Complement C3d | 3% |

No significant cross-reactivity observed with complement C1q, C1qBP, C1r, C1s, C2, C3, C3c, C4, C4BP, C5, C6, C7, C8, C8G, C9, Factor B, Factor D, Factor H, and Factor I proteins.

16. Species Reactivity

This kit recognizes human Complement Factor P.

| Species | Cross Reactivity (%) |
|---------|----------------------|
| Dog | None |
| Cow | None |
| Monkey | 70% |
| Mouse | None |
| Rat | None |
| Pig | None |
| Rabbit | None |

Please contact our Technical Support team for more information.

17. Troubleshooting

| Problem | Reason | Solution |
|---------------|---|---|
| Low Precision | Use of expired components | Check the expiration date listed before use. Do not interchange components from different lots |
| | Improper wash step | Check that the correct wash buffer is being used. Check that all wells are empty after aspiration. Check that the microplate washer is dispensing properly. If washing by pipette, check for proper pipetting technique |
| | Splashing of reagents while loading wells | Pipette properly in a controlled and careful manner |
| | Inconsistent volumes loaded into wells | Pipette properly in a controlled and careful manner. Check pipette calibration. Check pipette for proper performance |
| | Insufficient mixing of reagent dilutions | Thoroughly agitate the lyophilized components after reconstitution. Thoroughly mix dilutions |
| | Improperly sealed microplate | Check the microplate pouch for proper sealing. Check that the microplate pouch has no punctures. Check that three desiccants are inside the microplate pouch prior to sealing |

| | | |
|--|--|---|
| Unexpectedly low or high signal intensity | Microplate was left unattended between steps | Each step of the procedure should be performed uninterrupted |
| | Omission of step | Consult the provided procedure for complete list of steps |
| | Steps performed in incorrect order | Consult the provided procedure for the correct order |
| | Insufficient amount of reagents added to wells | Check pipette calibration. Check pipette for proper performance |
| | Wash step was skipped | Consult the provided procedure for all wash steps |
| | Improper wash buffer | Check that the correct wash buffer is being used |
| | Improper reagent preparation | Consult reagent preparation section for the correct dilutions of all reagents |
| | Insufficient or prolonged incubation periods | Consult the provided procedure for correct incubation time |

| | | |
|-------------------------------------|--|--|
| Deficient Standard Curve Fit | No-optimal sample dilution | <p>Sandwich ELISA: If samples generate OD values higher than the highest standard point (P1), dilute samples further and repeat the assay. Competitive ELISA: If samples generate OD values lower than the highest standard point (P1), dilute samples further and repeat the assay. User should determine the optimal dilution factor for samples</p> |
| | Contamination of reagents | <p>A new tip must be used for each addition of different samples or reagents during the assay procedure</p> |
| | Contents of wells evaporate | <p>Verify that the sealing film is firmly in place before placing the assay in the incubator or at room temperature</p> |
| | Improper pipetting | <p>Pipette properly in a controlled and careful manner. Check pipette calibration. Check pipette for proper performance</p> |
| | Insufficient mixing of reagent dilutions | <p>Thoroughly agitate the lyophilized components after reconstitution. Thoroughly mix dilutions</p> |

18. Notes

Technical Support

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