BioLegend®

Enabling Legendary Discovery™

Human Properdin

ELISA MAX™ *Deluxe Set*

Cat. No. 448904

BioLegend's ELISA MAX™ Deluxe Human Properdin ELISA Kit is a Sandwich Enzyme-Linked Immunosorbent Assay (ELISA), utilizing a monoclonal capture antibody and biotinylated monoclonal detection antibody specific for human Properdin. This set contains necessary reagents for the accurate quantification of natural and recombinant human Properdin in serum, plasma, urine and other biological fluids. Biolegend's ELISA MAX™ Deluxe Sets are cost-effective, sensitive, accurate, and robust.

It is highly recommended that this instruction sheet be read in its entirety before using this product. Do not use this set beyond the expiration date.

Materials Provided

1. Human Properdin ELISA MAX™ Capture Antibody (200X)
2. Human Properdin ELISA MAX™ Detection Antibody (200X)
3. Human Properdin Standard
4. Avidin-HRP (1,000X)
5. Substrate Solution D
6. Coating Buffer B (5X)
7. Assay Diluent B (5X)

Introduction

Properdin, known as CFP or complement factor P, is a proten that activates the complement regulatory proteins of the innate immune system. It is a glycoprotein produced by leukocytes and found in plasma. It positively regulates alternative pathway activity by stabilizing C3 and C5 convertases. Properdin's involvement in complement activity changes the cellular microenvironment, therefore contributing to both the innate and adaptive immune responses. This includes production of inflammatory cytokines, immune cell infiltration, antigen presenting cell maturation and tissue damage. As an inflammatory modulator, it can enhance damaging inflammation. Studies have shown its implication in several renal diseases, highlighting its role as a potential urinary biomarker. Its role in inflammatory conditions is multifacted, and as such, there is potential for the development of new disease therapies.

Principle of the Test

BioLegend's ELISA MAX™ Deluxe Set is a sandwich Enzyme-Linked Immunosorbent Assay (ELISA). A monoclonal anti-human Properdin antibody is first coated on a 96-well plate. Standards and samples are added to the wells, and Properdin binds to the immobilized capture antibody. Next, a biotinylated monoclonal anti-human Properdin detection antibody is added, producing an antibody-antigen-antibody "sandwich". Avidin-horseradish peroxidase is subsequently added, followed by TMB Substrate Solution, producing a blue color in proportion to the concentration of Properdin present in the sample. Finally, the Stop Solution changes the reaction color from blue to yellow, and the microwell absorbance is read at 450 nm with a microplate reader.

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

Materials to be Provided by the End-User

- Microwell plates: BioLegend Cat. No. 423501 is recommended
- Wash Buffer: BioLegend Cat. No. 421601 is recommended, or PBS + 0.05% Tween-20
- Stop Solution: BioLegend Cat. No. 423001 is recommended, or acid solution, e.g. 2N H_2SO_4
- Plate Sealers: BioLegend Cat. No. 423601 is recommended
- PBS (Phosphate-Buffered Saline): 8.0 g NaCl, 1.16 g Na_2HPO_4, 0.2 g KH_2PO_4, 0.2 g KCl, add deionized water to 1 L; pH to 7.4, 0.2 µm filtered
- Deionized (DI) water
- A microplate reader capable of measuring absorbance at 450 nm
- Adjustable pipettes to measure volumes ranging from 2 µL to 1 mL
- Wash bottle or automated microplate washer
- Log-Log graph paper or software for data analysis
- Tubes to prepare standard dilutions
- Timer

Storage Information

- Store kit components between 2°C and 8°C.
- After reconstitution of the lyophilized standard with 1X Assay Diluent B, aliquot into polypropylene vials and store at -70°C for up to one month. Avoid repeated freeze/thaw cycles.
- Prior to use, bring all components to room temperature (18°C-25°C). Upon assay completion, return all components to appropriate storage conditions.

Health Hazard Warnings

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details (www.biolegend.com/msds).
2. Substrate Solution D is harmful if ingested. Additionally, avoid skin, eye or clothing contact.
3. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

Specimen Collection and Handling

Urine: If necessary, centrifuge to remove debris prior to analysis. Samples can be stored at < -20°C. Avoid repeated freeze/thaw cycles.

Serum: Use a serum separator tube and allow clotting for at least 30 minutes, then centrifuge for 10 minutes at 1,000 X *g*. Remove serum layer and assay immediately or store serum samples at < -20°C. Avoid repeated freeze/thaw cycles. Serum specimens should be clear and non-hemolyzed.

Plasma: Collect blood samples in citrate, heparin or EDTA containing tubes. Centrifuge for 20 minutes at 1,000 x g within 30 minutes of collection. Assay immediately or store plasma samples at < -70°C. Avoid repeated freeze-thaw cycles.

Reagent and Sample Preparation

Do not mix reagents from different sets or lots. Reagents and/or antibodies from different manufacturers should not be used with this set. All reagents should be diluted immediately prior to use.

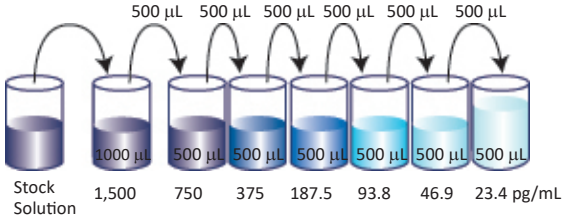
NOTE: Precipitation of 5X Assay Diluent B may be observed when stored long term between 2°C and 8°C. The precipitation does not alter the performance of the Buffer. If heavy precipitation is observed after the dilution to 1X Assay Diluent B, it can be filtered to clarify the solution.

Preparation of 1X Reagent for 1 Plate

Material	Dilute with
2.4 mL of Coating Buffer B (5X)	9.6 mL of Deionized Water
60 µL of Capture Antibody (200X)	12 mL of 1X Coating Buffer B
12 mL of Assay Diluent B (5X)	48 mL of PBS
60 µL of Detection Antibody (200X)	12 mL of 1X Assay Diluent B
12 µL of Avidin-HRP (1,000X)	12 mL of 1X Assay Diluent B

Lyophilized vials are under vacuum pressure. Reconstitute lyophilized standard by following the instructions described in Lot-Specific Certificate of Analysis/ELISA MAX™ Deluxe Set Protocol. Allow the reconstituted standard to sit for 15 minutes at room temperature, then mix gently prior to making dilutions.

Prior to use, prepare 1,000 µL of the top standard at a concentration of 1,500 pg/mL from the stock solution in 1X Assay Diluent B (**refer to Lot-Specific Certificate of Analysis/ELISA MAX™ Deluxe Set Protocol**). Perform six two-fold serial dilutions of the 1,500 pg/mL top standard with 1X Assay Diluent B in separate tubes. After diluting, the human Properdin standard concentrations are 1,500 pg/mL, 750 pg/mL, 375 pg/mL, 187.5 pg/mL, 93.8 pg/mL, 46.9 pg/mL, and 23.4 pg/mL, respectively. 1X Assay Diluent B serves as the zero standard (0 pg/mL).



Samples: In general, serum and plasma samples require a 15,000-fold dilution. Urine samples require no dilution. For other samples, the end user may need to determine the dilution factors in preliminary experiment. If dilutions are required, use 1X Assay Diluent B as the sample diluent.

Assay Procedure

Do not use sodium azide in any solutions as it inhibits the activity of the horseradish-peroxidase enzyme.

1. One day prior to running the ELISA, dilute Capture Antibody in 1X Coating Buffer B as described in Reagent Preparation. Add 100 µL/well of this Capture Antibody solution to the 96-well plate provided in this set. Seal plate and incubate overnight between 2°C and 8°C.
2. Bring all reagents to room temperature (RT) prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate. A standard curve is required for each assay.
3. Wash plate 4 times with at least 300 µL Wash Buffer per well and blot residual buffer by firmly tapping plate upside down on absorbent paper. **All subsequent washes should be performed similarly.**
4. To block non-specific binding and reduce background, add 200 µL 1X Assay Diluent B per well.
5. Seal plate and incubate at RT for 1 hour with shaking on a plate shaker (e.g. 500 rpm with a 0.3 cm circular orbit). All subsequent incubation with shaking should be performed similarly.

6. While plate is being blocked, prepare the appropriate sample dilutions (if necessary) and standards.
7. Wash plate 4 times with Wash Buffer.
8. Add 100 µL/well of standards or samples to the appropriate wells. If dilution is required, samples should be diluted in 1X Assay Diluent B before adding to the wells.
9. Seal plate, incubate at RT for 2 hour with shaking
10. Wash plate 4 times with Wash Buffer.
11. Add 100 µL/well of diluted Detection Antibody solution, seal plate and incubate at RT for 1 hour with shaking.
12. Wash plate 4 times with Wash Buffer.
13. Add 100 µL/well of diluted Avidin-HRP solution, seal plate and incubate at RT for 30 minutes with shaking.
14. Wash plate 5 times with Wash Buffer. For this final wash, soak wells in Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.
15. Add 100 µL/well Substrate Solution D and incubate **in the dark** for 10 minutes. Positive wells should turn blue in color. It is not necessary to seal the plate during this step.
16. Stop reaction by adding 100 µL/well of Stop Solution. Positive wells should turn from blue to yellow.
17. Read absorbance at 450 nm within 15 minutes. If the reader can read at 570 nm, the absorbance at 570 nm can be subtracted from the absorbance at 450 nm.

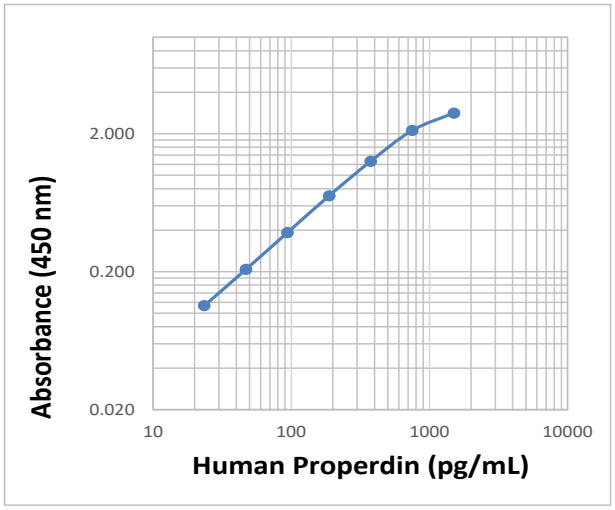
***Optimal substrate incubation time depends on laboratory conditions and the optical linear ranges of ELISA plate readers.**

Calculation of Results

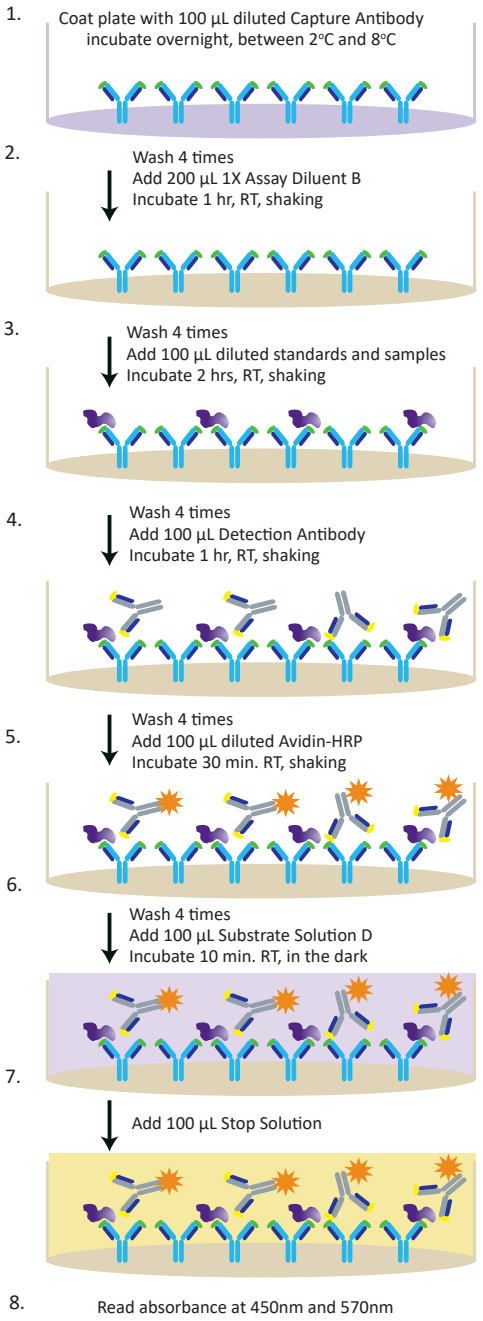
The data is best calculated with computer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. If the samples were diluted, multiply by the appropriate dilution factor. If a test sample's absorbance value falls outside the standard curve ranges, that test sample needs to be reanalyzed at a higher or lower dilution as appropriate.

Typical Data

Standard Curve: This standard curve was generated at BioLegend for demonstration purposes only. A standard curve must be run with each assay.



Assay Procedure Summary



Performance Characteristics

Sensitivity: The expected minimum detectable concentration of human Properdin for this set is 7.21 pg/mL.

Specificity: No cross reactivity was observed when this kit was used to analyze multiple human recombinant proteins, including CFD, R-Spondin 1, Thrombospondin-2, and C6.

Troubleshooting

High Background:

- Background wells were contaminated.
- Matrix used had endogenous analyte.
- Plate was insufficiently washed.
- TMB Substrate Solution was contaminated.

No signal:

- Incorrect or no antibodies were added.
- Avidin-HRP was not added.
- Substrate solution was not added.
- Wash buffer contains sodium azide.

Low or poor signal for the standard curve:

- Standard was incompletely reconstituted or was stored improperly.
- Reagents were added to wells with incorrect concentrations.
- Plate was incubated with improper temperature, timing or agitation.

Signal too high, standard curves saturated:

- Standard was reconstituted with less volume than required.
- One or more reagent incubation steps were too long.
- Plate was incubated with inappropriate temperature, timing, or agitation.

Sample readings out of range:

- Samples contain no or below detectable levels of analyte.
- Samples contain analyte concentrations greater than highest standard point.

High variations in samples and/or standards:

- Pipetting errors may have occurred.
- Plate washing was inadequate or non-uniform.
- Samples were not homogenous.
- Samples or standard wells were contaminated.

BioLegend, Inc.

BioLegend is ISO 13485 Certified

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