Application Note

Complement Factor P Quantitative Assay

Doc. no. LABEL-DOC-0628 3.0

Effective Date: 13-May-2025

For research use only. Not for use in diagnostic procedures.

This application note contains a suggested protocol and pertaining performance data.

Each individual laboratory must set up their own method and perform relevant validations.

FOR REFERENCE USE ONLY

REF **COMPL FPQ RUO**

Σ 96

1 PURPOSE

The purpose of the product is to quantitate the amount of human Factor P in test samples.

The product is intended for use by trained laboratory personnel. The results shall not be used for clinical diagnosis or patient management. FOR RESEARCH USE ONLY.

2 BACKGROUND

Factor P (Complement P, Properdin, FP) is a positive regulator and an initiator of the alternative pathway (AP) for complement activation. It binds surface-bound C3 and C5 convertases and stabilizes them to amplify the activation cascade[1]. Factor P binding increases the half-life of the convertase complex approximately 10-fold[2]. It is suggested, however debated, that Factor P also can initiate complement activation by binding for example cell surfaces or certain biological substrates, recruiting C3b or C3(H_2O) and Factor B and thus initiate the AP pathway[1,3]. Factor P opposes the negative regulation of Factor H that enhances the dissociation of C3b and Bb and mediates Factor I cleavage of C3b to the inactive iC3b[3] (Figure 1).

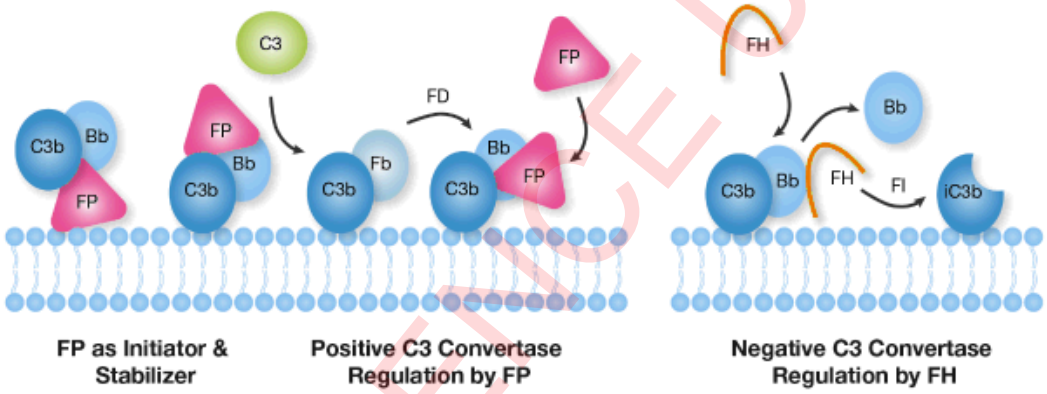


Figure 1: Factor P as stabilizer and initiator of the alternative complement pathway (left) and the opposing negative regulation by Factor H[3] (right).

Factor P is not produced in hepatocytes as most complement proteins, but instead by several cell types including monocytes, macrophages, T-cells and granulocytes. It is likely that transient increased concentration of Factor P enhances the AP upon local stimuli[4]. For example, neutrophils have Factor P-containing granules that are secreted upon stimulation and can enhance the platelet-granulocyte aggregate formation[1].

In plasma, Factor P is present in a concentration of approximately 4-25 µg/mL[5]. Factor P is an elongated 53 kDa glycoprotein that oligomerizes *in vivo* to dimers, trimers or tetramers (P_2, P_3 and P_4) in a ratio of 26:54:20 (P_2:P_3:P_4) in head to tail structures [5,6].

Mutations, deficiencies, protein levels as well as protein deposits of Factor P are connected to diseases and disorders summarized by Chen *et al* [3]. Deficiencies generally increase the susceptibility for meningococcal disease and other infectious diseases[7]. Altered serum levels have been associated with for example C3 glomerulopathy, Lupus Nephritis, sepsis and chronic heart failure and IgA nephropathy[8].

FOR REFERENCE USE ONLY

3 PRINCIPLE OF COMPLEMENT FACTOR P QUANTITATIVE ASSAY

3.1 Principle of complement Factor P Quantitative Assay

The Factor P Quantitative Assay is a sandwich ELISA for quantifying human Factor P in, for example, serum or plasma samples. The assay is based on immobilized antibody in a 96-well plate, which captures Factor P present in test samples incubated in the plates. The bound Factor P is detected with a secondary antibody labelled with HRP. The HRP subsequently catalyzes a cleavage of the substrate that induces a color change correlating with the concentration of Factor P in the test sample.

3.2 General assay information

GENERAL ASSAY INFORMATION	
Working time:	2 hours
Concentration range	0-200 ng/ml
Sample matrix	Plasma and serum have been tested
Working volume	100 µl/well
Number of samples	40 samples in duplicate
Species	Human FP. Other not tested
Working temperature	Room temperature (20-25°C)

4 WARNINGS, AND PRECAUTIONS

FOR RESEARCH USE ONLY. Not for use as a diagnostic tool or in the management of patients.

- Serum or plasma samples which are icteric, lipemic or hemolyzed may give erroneous results.
- The kit contains potentially infectious material. Calibrator, High control and Low control contain plasma which is derived from human. Although tested against and confirmed negative for hepatitis B, hepatitis C and antibodies for HIV, this material must be treated as potentially infectious.
- Never pipette reagents or test samples by mouth. In case of exposure of skin or eyes to reagents or test samples, flush with plenty of water.
- The Conjugate Solution contains 2-methyl- 2H-isothiazol-3-one [EC no. 220-239-6]. It may cause an allergic skin reaction.
- The TMB substrate (3, 3', 5, 5'-tetramethylbenzidine) is toxic by inhalation, in contact with skin and if swallowed. Be careful when handling the substrate.
- Safety data sheet for all hazardous components contained in this kit is available from Svar Life Science on request.



WARNING

Wash Buffer and Conjugate:
Contains: Reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H-isothiazol-3-one [EC no. 220-239-6] (3:1)
H317 May cause an allergic skin reaction

FOR REFERENCE USE ONLY

H412 Harmful to aquatic life with long lasting effects.
P261 Avoid breathing spray.
P273 Avoid release to the environment.
P280 Wear protective gloves.
P333 + P313 If skin irritation or rash occurs: Get medical advice/attention.

Calibrator, High control, Low control, Dilution buffer:

EUH208 Contains "Reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H-isothiazol-3-one [EC no. 220-239-6] (3:1)" May produce an allergic reaction.
EUH210 Safety data sheet available on request.

Stop Solution:

EUH210 Safety data sheet available on request.

5 KIT CONTENTS

- One frame with microtiter wells (12x8) coated with anti-Factor P monoclonal antibody, sealed in a foil pack with a desiccation bag.

- 3.0 mL vial with Calibrator (200 ng/mL). Ready to use. The Calibrator must be further diluted according to instructions in 9.1.2.

- 1.5 mL Low control (LC), green color, green cap. Ready to use.

- 1.5 mL High control (HC), red color, red cap. Ready to use.

- 2x32 mL Diluent (Dil), red color. Ready to use.

- 30 mL Wash solution, 30x concentrated

- 15 mL Conjugate containing HRP-labelled antibody directed against Factor P, blue color, brown vial. Ready to use.

- 15 mL Substrate TMB, brown vial. Ready to use.

- 15 mL Stop solution (0.5M H_2SO_4). Ready to use.

Please note:

- See instructions for handling of reagents in section 9.
- Components from different lots shall not be mixed.

6 MATERIALS OR EQUIPMENT NEEDED BUT NOT PROVIDED

- Microplate reader with filter 450 nm and 620 nm
- Orbital shaker (unless included in the function of the microplate reader)
- Precision pipettes with disposable tips
- Washer for 96-well plates or strips
- Absorbent tissue
- Vessels for reagent and sample dilution
- Timer

7 STABILITY AND STORAGE

The reagents should be stored at 2-8°C. When stored at 2-8°C, the diluted Wash solution is stable until the date of expiration for the kit.

FOR REFERENCE USE ONLY

8 SPECIMEN COLLECTION AND PREPARATION

Whole blood samples are to be collected using aseptic venipuncture technique, and serum or EDTA plasma obtained using standard procedures. It is recommended to draw a minimum of 5 mL of whole blood per sample. Centrifuge blood samples and transfer cell-free serum or plasma to a clean tube.

The centrifuged serum or EDTA plasma may be kept at 4°C. For longer storage, serum and plasma specimen should be frozen at -20°C or lower. It is recommended that test samples are not frozen and thawed more than twice before analysis.

Each laboratory should determine the acceptability of the test sample storage conditions as this may vary due to preanalytical factors.

9 PROCEDURE

9.1 Preparation and handling of reagents

Equilibrate reagents (Microtiter plate, Calibrator, Controls, Diluent, Wash solution, Conjugate, Substrate and Stop solution) to room temperature (20-25°C).

9.1.1 Dilution of Wash buffer

Dilute the Wash buffer 30x with deionized water.

Example: To 10 mL of Wash buffer concentrate, add 290 mL of deionized water. Mix well.

9.1.2 Dilution of Factor P Quantitative Calibrator

Dilute the Calibrator with Diluent to the following concentrations using a 1:2 serial dilution (mix thoroughly in all dilution steps):

200 ng/mL (undiluted), 100 ng/mL, 50ng/ml, 25 ng/mL and 12.5 ng/mL

The Diluent is used as assay blank (0 ng/mL).

9.2 Assay procedure

1. Equilibrate reagents (Microtiter plate, Calibrator, Controls, Diluent, Wash solution, Conjugate, Substrate and Stop solution) to room temperature (20-25°C).
2. Dilute the Wash solution 30x as stated in section 9.1.1.
3. Thaw test samples at room temperature.
4. Dilute the Calibrator as stated in section 9.1.2.
5. Dilute samples 1/200* in Diluent (ex. 10 µl test plasma + 1990 µl diluent). Mix all diluted samples gently but thoroughly.

   *Other dilutions may be used but need to be tested and verified by the user. Adjust calculations correspondingly.
6. Transfer 100µL diluted sample, Calibrator dilutions, blank and Controls to duplicate wells in the microtiter plate and incubate at room temperature for 60 minutes.

   Note: Transfer of samples, Calibrators and Controls to the plate should not take more than **10 minutes** from the first to the last well to avoid drift of signal.

FOR REFERENCE USE ONLY

7. Wash 3 times with 300 µl diluted Wash solution by filling and emptying the wells.
8. Add 100 µl Conjugate to the wells. Incubate at room temperature for 30 minutes.
9. Wash 3 times with 300 µl diluted Wash solution by filling and emptying the wells.
10. Add 100 µl Substrate to the wells. Incubate at room temperature for 30 minutes.
11. Add 100 µl stop solution to the wells.
12. Mix the microtiter plates gently on an orbital shaker (or similar) for a few seconds.
13. Read absorbance at 450 nm om the microplate reader. Read at 620 nm as reference wavelength.

9.3 Disposal of kit contents

Kit material and waste from the analysis should be disposed of as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or companies dealing with waste management in your region, who will be able to advice you on the disposal of hazardous waste.

10 EVALUATION OF RESULTS

For each datapoint, subtract the 620 nm reference wavelength from the 450 nm wavelength and calculate mean absorbance values for all duplicate samples.

Construct a calibrator curve by plotting the absorbance of the six calibrators against the concentrations: 0 ng/ml, 12.5 ng/ml, 25 ng/ml, 50 ng/ml, 100 ng/ml and 200 ng/ml.
A 4-parameters curve fit was used in the example of this Application Note (Figure 2), but users may select a different curve fit. Read the concentrations of the unknown samples against the calibrator curve.

Note: To calculate the concentration of Factor P in the sample, compensation for dilution must be performed i.e. 200x according to the protocol above. If a different sample dilution is chosen, compensation for this should be done correspondingly.

FOR REFERENCE USE ONLY

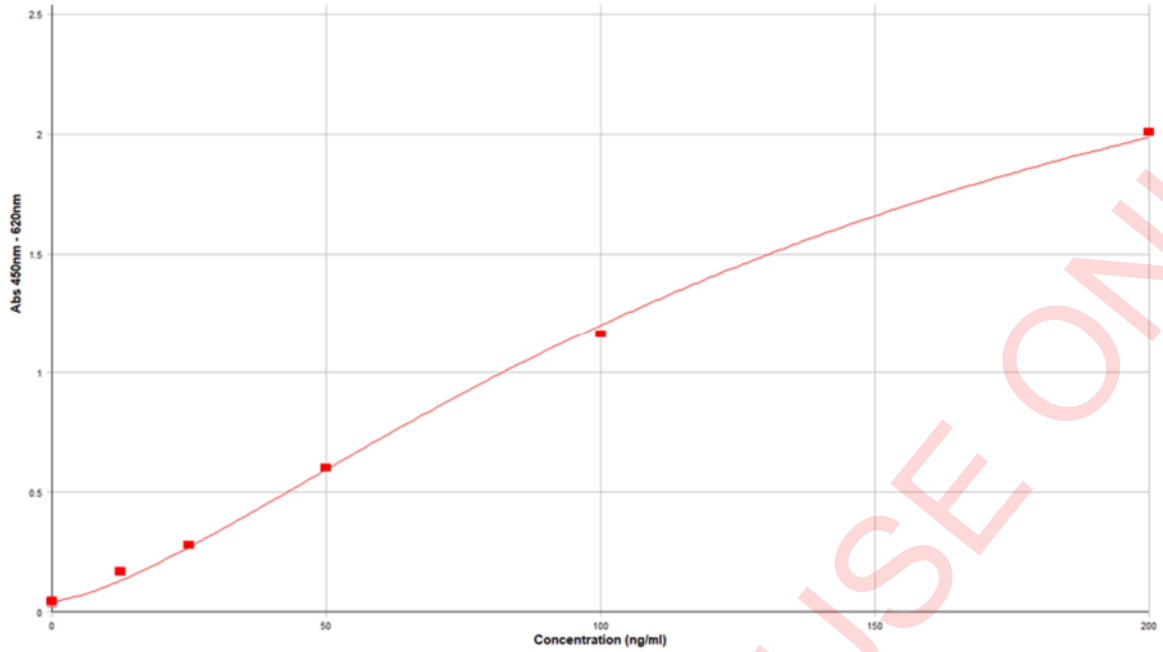


Figure 2. Example of a calibrator curve

Please Note: The figure above shows an example of a standard curve and should not be used for actual sample interpretation.

11 QUALITY CONTROL

Absorbance for Calibrator shall be >1.0

The high and low controls are intended to monitor for substantial reagent failure. If the test result for any of the controls are not within their respective limit range the test should be considered as invalid and should be repeated in its entirety. Limits are found on the certificate included in the kit.

12 PERFORMANCE CHARACTERISTICS

A summary of assay performance of the product is given below. The performance data has been obtained using serum test samples. Other sample matrices could potentially display a different performance.

12.1 Reference range

Reference range: 6.9-18.5 µg/mL

The reference range is based on the 2.5 – 97.5 percentiles of the ranked Factor P values obtained from 120 blood donor samples in the Factor P Quantitative assay.

12.2 Precision

12.2.1 Intra-assay variability

The individual coefficients of variation (CV) intra-assay for 9 samples analyzed in 8 replicates were between 1 and 5%.

12.2.2 Inter-assay variability

The individual coefficients of variation (CV) inter-assay for 9 samples analyzed in 8 replicates on three separate occasions were between 1 and 6%.

FOR REFERENCE USE ONLY

12.3 Lot-to-lot variability

Individual variability for 9 samples analyzed in 8 replicates at three occasions on two individual kit batches ranged from 0 to +27%.

13 LIMITATIONS

This kit has been tested for use with human EDTA plasma and serum only. Other test matrices and species have not been tested.

This kit is for research use only. It is not intended for use as a diagnostic tool or in the management of patients.

FOR REFERENCE USE ONLY

14 TROUBLESHOOTING

Product problem/failure: suggested possible causes and recommended action for resolution.

Problem	Possible causes	Solution
Calibrator or control values out of range	Incorrect temperature, timing or pipetting, reagents are not mixed	Check that the time and temperature were correct. Repeat test.
	Cross contamination of controls	Pipette carefully
	Optical pathway is not clean.	Check for dirt or air-bubbles in the wells. Wipe plate and reread.
All test results negative	One or more reagents are not added or added in wrong sequence.	Recheck procedure. Check for unused reagents. Repeat test.
	Antigen coated plate is inactive	Check for obvious moisture in unused wells. Wipe plate bottom and reread.
All samples including negative control give high and even test results.	Contaminated buffers or reagents.	Check all solutions for turbidity.
	Washing solution is contaminated.	Use clean container. Check the quality of water used for preparation of solution.
	Inappropriate dilution of test sample.	Repeat test.
Poor precision.	Pipette delivery CV >5% or samples not mixed.	Check the calibration of pipette. Use reproducible technique. Avoid air bubbles in pipette tip.
	Samples or reagents are not sufficiently mixed or not equilibrated to room temperature.	Mix all reagents gently but thoroughly and equilibrate to room temperature.
	Reagent addition is taking too long time, inconsistency in timing intervals.	Develop consistent uniform technique and use multi-tip device or auto-dispenser to decrease time.
	Optical pathway not clean.	Check for air bubbles in the wells. Wipe plate bottom and reread
	Washing not consistent; trapped bubbles, washing solution left in the wells.	Check that all wells are filled and aspirated uniformly. Dispense liquid above level of reagent in the well. After last wash, empty the wells by tapping the strip on an absorbent tissue.

FOR REFERENCE USE ONLY

15 LITERATURE REFERENCES

(1) Blatt, A. Z.; Pathan, S.; Ferreira, V. P. Properdin: A Tightly Regulated Critical Inflammatory Modulator. *Immunol Rev* **2016**, *274* (1), 172–190. https://doi.org/10.1111/imr.12466.
(2) Fearon, D. T.; Austen, K. F. Properdin: Binding to C3b and Stabilization of the C3b-Dependent C3 Convertase. *J Exp Med* **1975**, *142* (4), 856–863.
(3) Chen, J. Y.; Cortes, C.; Ferreira, V. P. Properdin: A Multifaceted Molecule Involved in Inflammation and Diseases. *Mol. Immunol.* **2018**, *102*, 58–72. https://doi.org/10.1016/j.molimm.2018.05.018.
(4) Cortes, C.; Ohtola, J. A.; Saggu, G.; Ferreira, V. P. Local Release of Properdin in the Cellular Microenvironment: Role in Pattern Recognition and Amplification of the Alternative Pathway of Complement. *Front Immunol* **2013**, *3*. https://doi.org/10.3389/fimmu.2012.00412.
(5) Pangburn, M. K. Analysis of the Natural Polymeric Forms of Human Properdin and Their Functions in Complement Activation. *The Journal of Immunology* **1989**, *142* (1), 202–207.
(6) Smith, C. A.; Pangburn, M. K.; Vogel, C. W.; Müller-Eberhard, H. J. Molecular Architecture of Human Properdin, a Positive Regulator of the Alternative Pathway of Complement. *J. Biol. Chem.* **1984**, *259* (7), 4582–4588.
(7) Fijen, C. A. P.; van den Bogaard, R.; Schipper, M. Properdin Defciency: Molecular Basis and Disease Association. *Molecular Immunology* **1999**, *36*, 863–867.
(8) Michels, M. A. H. M.; Volokhina, E. B.; van de Kar, N. C. A. J.; van den Heuvel, L. P. W. J. The Role of Properdin in Complement-Mediated Renal Diseases: A New Player in Complement-Inhibiting Therapy? *Pediatr Nephrol* **2019**, *34* (8), 1349–1367. https://doi.org/10.1007/s00467-018-4042-z.

FOR REFERENCE USE ONLY

16 DESCRIPTION OF SYMBOLS

LOT	*Batch number.*
REF	*Catalog number.*
	*Use-by-date.*
	*Temperature limit.*
	*Biological risk.*
	*Consult instructions for use.*
	*Manufacturer.*
	*Contents sufficient for 96 tests.*
	*Warning.*
Ab	*Antibody (coated plate).*
DIL	*Diluent.*
BUF | WASH | 30X	*Wash buffer, 30x concentrate.*
H_2SO_4 | 0.5M	*Sulfuric Acid, 0.5 molar (stop solution).*
CONJ	*Conjugate.*

FOR REFERENCE USE ONLY

SUBS TMB	*Substrate TMB.*
CAL	*Calibrator.*
CONTROL H	*High control (HC).*
CONTROL L	*Low control (LC).*

FOR REFERENCE USE ONLY





www.svarlifescience.com

Svar Life Science AB
Visiting address:
Lundavägen 151
SE-212 24 Malmö, Sweden

Postal address:
P.O. Box 50117 SE-202 11 Malmö, Sweden