1 PURPOSE

The purpose of the product is to determine the function of Factor P in test samples. The functionality is determined qualitatively in relation to the calibrator (human serum) set to be 100% functional.

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]. The oligomeric state correlates with C3 convertase stabilizing function and distribution of oligomers affects the overall Factor P functionality[7].

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[8]. Altered serum levels have been associated with for example C3 glomerulopathy, Lupus Nephritis, sepsis and chronic heart failure and IgA nephropathy[9].

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3 PRINCIPLE OF COMPLEMENT FACTOR P FUNCTIONAL ASSAY

3.1 Principle of complement Factor P Functional Assay

The Factor P Functional assay is an enzyme-linked immunosorbent assay (ELISA) that combines principles of the functional complement activity assays with the use of labelled antibodies specific for deposited complement proteins. The amount of deposited complement proteins is proportional to the functional activity of Factor P in the sample.

3.2 General assay information

GENERAL ASSAY INFORMATION	
Working time:	4 hours
Sample matrix	Plasma and serum
Working volume	50 µl/well and 20 µl/well
Number of samples	46 samples in duplicate
Species	Human FP. Other not tested
Working temperature	37°C and 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 human material. Calibrator and Activator contain plasma 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 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 upon request.

WARNING

Wash Buffer and Conjugate:
Contains ProClin 300: 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.
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.

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Calibrator, Negative Control, Dilution Buffer activator, Dilution Buffer samples, Quench:

EUH208: Contains ProClin 300: 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 upon 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.
- 1.5 mL Negative control (NC), green color, green cap. Ready to use.
- 3.0 mL Calibrator, 200 ng/mL. Ready to use.
- 32 mL Diluent (Dil), red color. Ready to use.
- 30 mL Wash solution, 30x concentrated
- Activator, lyophilized.
- 15 mL Activator Diluent. Ready to use.
- 3.0 mL Quench. Ready to use.
- 7.5 mL Conjugate containing HRP-labelled antibody directed against C3b, 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 (200 rpm)
- Precision pipettes with disposable tips
- Sealing film for 96-well plates or strips
- Washer for 96-well plates or strips
- Absorbent tissue
- Vessels for reagent and sample dilution
- Ice or equivalent plate cooling device
- Timer

7 STABILITY AND STORAGE

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

Lyophilized and reconstituted Activator should be stored at **-70°C** or below. The reconstituted activator may be thawed once.

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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.

Determine concentration of Factor P in each test sample before performing the Complement P Functional Assay analysis (see section 10 “Assay procedure” step 5). For this determination of Factor P concentration, the Factor P Quantitative Assay (product code COMPL FPQ RUO), from Svar Life Sciences AB can be used.

9 PROCEDURE

Note: Before running the functional Factor P assay, Factor P concentrations should be quantitatively determined in each individual sample.

9.1 Preparation and handling of reagents

9.1.1 Equilibration of reagents

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

Keep activator diluent refrigerated until dilution of the Activator.

9.1.2 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 thoroughly.

9.1.3 Reconstitution of lyophilized Activator (just before use)

Gently tap down all lyophilized material to the bottom of the vial and remove the cap. Add 400 µL deionized water directly to the lyophilized material. Replace the cap. Allow the vial to stand on ice for 5 minutes and then gently shake or vortex occasionally until completely dissolved.

Lyophilized and reconstituted activator should be stored at **-70°C** or below and may be thawed once. Thaw reconstituted and frozen activator on ice before use.

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9.2 Assay procedure

1. Equilibrate reagents (Microtiter plate, Calibrator, Negative Control, Diluent, Quench, Wash solution, Conjugate, Substrate and Stop solution) to room temperature (20°C-25°C). Activator Diluent should be kept refrigerated on ice or at 2-8°C.

2. Dilute the wash solution 30x (see section 9.1.1).

3. Thaw test samples at room temperature.

4. Dilute all test samples in Diluent to a Factor P concentration corresponding to 200 ng/mL. Mix all diluted samples gently but thoroughly.

5. Transfer 50µL diluted sample, calibrator, and negative control to the microtiter wells in duplicates and seal the plate with plastic film. Incubate at 37°C for 60 minutes. **Note:** transfer of samples, calibrators and control to the plate should not take more than 15 minutes from the first to the last well to avoid drift of signal.

6. Wash 3 times with 150 µl diluted wash solution by filling and emptying the wells.

7. Just before use, reconstitute the Activator using 400 µL of deionized water per vial, see section 9.1.2. Dilute the reconstituted Activator with **cold** Activator diluent according to Certificate of Analysis. Mix the diluted Activator gently. Keep the diluted activator on ice until addition to the plate.

8. Add 50 µL of diluted Activator per well and seal the plate with plastic film. Incubate at 37°C for 90 minutes.

9. Add 20 µl Quench per well. Incubate at plate shaker (200 rpm) in room temperature for 60 seconds.

10. Wash 3 times with 150 µl diluted wash solution by filling and emptying the wells.

11. Add 50 µl conjugate to the wells and seal the plate with plastic film. Incubate at 37°C for 60 minutes.

12. Wash 3 times with 150 µl diluted wash solution by filling and emptying the wells.

13. Add 50 µl substrate to the wells. Incubate at room temperature for 30 minutes.

14. Add 50 µl stop solution to the wells.

15. Mix the microtiter plates gently on an orbital shaker (or similar) for a few seconds.

16. 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.

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10 EVALUATION OF RESULTS

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

Calculate the % complement activity as follows:

(sample - NC)/(Cal - NC)x100

Sample = Mean OD value for test sample
NC = Mean OD value for Negative Control
Cal = Mean OD value of Calibrator

11 QUALITY CONTROL

Specifications for Calibrator and Negative Control are stated on the Certificate of Analysis.

The OD of the Calibrator and the Negative Control monitor for assay or reagent failure. If the measured OD for the Calibrator or the Negative Control is not within their respective limit range, the test should be considered as invalid and should be repeated in its entirety.

12 PERFORMANCE CHARACTERISTICS

A summary of the 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: 89.6 – 176.6 % activity

The reference range is based on the 2.5 – 97.5 percentiles of the ranked Factor P activity values obtained from 120 blood donor samples in the Complement Factor P Functional 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 3 and 13%.

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 2 and 16%.

12.3 Lot-to-lot variability

Individual variability for 9 samples analyzed in 8 replicates on two individual kit batches ranged from 2 to 12%.

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.

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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 pipettes. Use reproducible technique. Avoid air bubbles in pipette tip.
	Plasma 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 the last wash, empty the wells by tapping the strip on an absorbent tissue.

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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.*

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QUE	*Quench.*
SUBS TMB	*Substrate TMB.*
CAL	*Calibrator.*
CONTROL -	*Negative control.*
ACT LYO	*Activator.*
DIL ACT	*Activator Diluent.*

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