

# *PROPERDIN ELISA KIT*



## Properdin ELISA Kit (KIT 033)

For the *in vitro* determination of properdin in human serum or plasma

April 2005

Batch: PP-0401CE

### INTENDED USE

To diagnose type-1 and type-2 properdin deficiency in patients and their blood relatives in cases of recurrent meningococcal infection, recurrent bacterial meningitis, time-separated clustering of meningococcal infections within a family, and meningococcal infections due to unusual serogroups.

### CLINICAL SIGNIFICANCE

Properdin, or factor P, is a plasma glycoprotein composed of oligomers of identical 52-55 kDa protein chains. Each chain consists of six thrombospondin repeat sequences between short N- and C-terminal domains.<sup>1</sup> Properdin is normally found as a mixture of head-to-tail dimers, trimers and tetramers, with a preponderance of trimers.<sup>2</sup> The protein is produced by a variety of leukocytes, including monocytes,<sup>3</sup> T lymphocytes<sup>4</sup> and neutrophils,<sup>5</sup> and also by endothelial cells in which properdin synthesis and release are induced by shear stress.<sup>6</sup>

Properdin participates in the alternative pathway of complement activation together with C3 and factors B, D, I and H. It stabilizes the labile C3bBb which is deposited on immune complexes or foreign surfaces. This permits amplification of C3bBb formation in competition with catabolism of C3b by factor I, which uses factor H as a cofactor. The local amplification process leads to the creation of the alternative pathway C5 convertase, C3bBb3b, and initiates the terminal pathway of complement activation.

Properdin prolongs the half-life of surface-bound C3bBb from 1½ minute to about 18 minutes. This is due to several effects: inhibition of C3b cleavage by factor I, increased affinity for factor B and inhibition of the dissociation of C3bBb into C3b and Bb. Properdin is consumed by binding to C3bBb, this binding showing an order of preference of tetramers over trimers over dimers, which is also the order of functional activity of the oligomeric forms.<sup>2</sup> Deficiency or malfunction of the molecule may lead to severe impairment of alternative pathway activation, depending on the precise nature of the defect. One parameter of functional defect in the presence of measurable levels of protein is an impaired generation of the more active tetramer and trimer forms.

### Properdin deficiency

The properdin gene is located on the short arm of the X chromosome,<sup>7</sup> and congenital properdin deficiencies are therefore inherited as typical X-linked recessive traits. Three types of familial deficiency have been described: type 1 (or I) is characterized by serum with very low or absent properdin activity in hemolytic assays and <0.1 µg/ml immunoreactive protein,<sup>8</sup> type 2 (or II) is characterized by low but detectable levels of immunoreactive protein (≈2 µg/ml) and impairment of

some, but not all functional tests;<sup>8,9</sup> and type 3 (or III) has normal levels of immunoreactive but dysfunctional protein.<sup>10</sup> Normal serum or plasma levels of properdin are variously quoted as about 5 or 25 µg/mL. These figures come from different laboratories and the absence of accepted standard preparations means that there is as yet no general consensus about the normal range e.g. for a Caucasian population. Female carriers show "Lyonization", i.e. the random suppression of one or other X chromosome in somatic cells, so that they may have low, medium or near-normal levels of immunoreactive properdin.

Different point mutations have been identified as being responsible for the different types of properdin deficiency. Type-1 deficiencies are due to mutations giving rise to premature stop codons in early exons<sup>11-13</sup> or to conserved amino-acid substitutions in later exons, thought to impair secretion and promote intracellular catabolism of the molecule.<sup>13</sup> Type-2 deficiencies are due to amino-acid substitutions that appear to be associated with defective oligomerization and increased extracellular breakdown.<sup>14</sup> Type-3 deficiency in one family was associated with a Tyr→Asp substitution at position 387 which did not affect oligomerization but impaired function, presumably by affecting binding to C3b.<sup>15</sup>

Properdin deficiency may also be secondary to consumption, which can occur as a result of factor H deficiency, a rare autosomal recessive trait.<sup>16</sup> This leads to spontaneous *in vivo* activation of the alternative pathway, with consumption of C3, factor B and terminal pathway components as well as properdin.

### Clinical correlates

Properdin deficiency of whatever type is associated with enhanced susceptibility to meningococcal disease. It was the occurrence of fulminant meningococcal infections in three members of a family that led to the first description of properdin deficiency.<sup>17</sup> Case clustering of meningococcal infections in families affected by properdin deficiency is not just due to contact: the individual infections may be dispersed in time and caused by meningococci of uncommon serogroups. However, groups of patients with unselected meningococcal disease show only modest percentages of complement deficiency, e.g. 3% in a Dutch study,<sup>18</sup> 11% in an Israeli study.<sup>19</sup> The frequency of heritable complement deficiencies, including properdin deficiency, in unselected meningococcal disease may vary with ethnicity: the Israeli study showed a high frequency, with properdin deficiency accounting for 2 of the 11 cases of complement deficiency.

The frequency of complement deficiencies including properdin deficiency rises markedly when

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cases of meningococcal disease are selected for unusual features. A Danish study found complement deficiencies in 14% of cases in which there were two or more infections within a family at an interval of >30 days, in 41% of cases that had suffered an additional episode of meningococcal infection or purulent meningitis of other etiology, and in 19% of cases due to uncommon, presumably low-virulent meningococcal serogroups such as W-135, 29E, X and Y.<sup>20</sup> Taken together these cases showed a predominance of defects of the initiation pathways, properdin deficiency being the most common. In the Dutch study,<sup>18</sup> 33% of patients with meningococcal infections due to uncommon serogroups had complement deficiency, the vast majority being over 5 years of age. 27% of complement-deficient relatives found by screening had had meningococcal disease, this figure being 18% in properdin-deficient (male) relatives.

When families are screened for properdin deficiency, it is typically found that only a proportion (about 1/5) of properdin-deficient males have actually had meningococcal disease. This raises the question of which additional factors might affect susceptibility. Chance exposure presumably plays a part, but concurrent immune defects may also be involved. In a Swiss family only 2 of 9 properdin-deficient males had meningococcal disease. These were distinguished from the others by lack of the IgG2m(n) allotype marker.<sup>12</sup> Fcγ receptor allotypes, affecting the phagocytosis of meningococci, were found to be significant in another study.<sup>21</sup> Mannan-binding lectin (MBL) deficiency, inherited as an autosomal dominant or co-dominant, is an independent risk factor for meningococcal disease,<sup>22</sup> and concurrent MBL deficiency, affecting around 12% of some populations, may increase susceptibility to meningococcal infection in properdin deficiency.

### Screening for properdin deficiency

The clinical correlates justify the determination of serum or plasma properdin levels in the individuals or groups defined above under "INTENDED USE".

Such testing will identify males affected by properdin deficiency of types 1 and 2, and those female carriers who have significantly reduced properdin levels, whose children should also be tested.

Properdin-deficient individuals should then be considered for additional tests such as the determination of IgG and Fcγ receptor allotypes, and serum levels of functional or oligomerized MBL. They should also be considered for vaccination against meningococcal disease.

### PRINCIPLE OF THE ASSAY

The assay is a sandwich ELISA performed in microwells coated with a monoclonal antibody against human properdin. Bound properdin is detected with the same monoclonal antibody labeled with biotin and the assay is developed with horseradish peroxidase (HRP)-conjugated streptavidin and a color-forming substrate. The assay is a 4-step procedure:

**Step 1.** Aliquots of calibrators, diluted serum or plasma samples and any controls are incubated in microwells precoated with monoclonal capture antibody directed against human properdin. Properdin present in the solutions will bind to the coat, while unbound material is removed by washing.

**Step 2.** Biotinylated monoclonal detection antibody is added to each test well and incubated. The detection antibody attaches to bound properdin, provided that properdin dimers or higher oligomers are present, while unbound detection antibody is removed by washing.

**Step 3.** HRP-conjugated streptavidin is added to each test well and allowed to form a complex with the bound biotinylated antibody. Unbound conjugate is removed by washing.

**Step 4.** A color-forming peroxidase substrate containing tetramethylbenzidine (TMB) is added to each test well. The bound HRP-streptavidin reacts with the substrate to generate a colored product. The enzymatic reaction is stopped chemically, and the color intensity is read at 450 nm in an ELISA reader. The color intensity (optical density) is a function of the concentration of properdin originally added to each well. The results for the calibrators are used to construct a calibration curve from which the concentrations of properdin in the test specimens are read.

### KIT COMPONENTS

Item	Contents	Quantity
1	12 x 8 Microwells + frame (ready to use)	1 plate
2	Sample Diluent (ready to use)	2 x 30 mL
3	Calibrators, 1 – 8 (ready to use) 0,5, 10, 20, 50, 100, 200, 500 mU/mL	8 x 1 mL
4	25 x Wash Solution Concentrate	1 x 30 mL
5	Biotinylated Properdin Antibody (ready to use)	1 x 12 mL
6	HRP-Streptavidin (ready to use)	1 x 12 mL
7	TMB Substrate (ready to use)	1 x 12 mL
8	Stop Solution (ready to use)	1 x 16 mL

**Note:** Liquid reagents contain the preservatives azide, thimerosal or Bronidox L.

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### MATERIALS REQUIRED BUT NOT PROVIDED

1. Adjustable micropipettes covering the range 1-1000 µL and corresponding disposable pipette tips
2. Polypropylene tubes to contain up to 1000 µL
3. Tube racks
4. Adjustable 8- or 12-channel micropipette (50-250 µL range) or repeating micropipette (optional).
5. Clean 1 L graduated cylinder
6. Deionized or distilled water
7. Cover for microplate
8. Clean container for diluted Wash Solution
9. Apparatus for filling wells during washing procedure (optional)
10. Lint-free paper towels or absorbent paper
11. Disposable pipetting reservoirs
12. Timer (60-minute range)
13. Calibrated ELISA plate reader capable of reading at 450 nm (preferably subtracting reference values at 650 or 620 nm)
14. Sodium hypochlorite (household bleach 1:10 dilution) for decontamination of specimens, reagents, and materials
7. The Stop Solution contains 0.5 M sulfuric acid and can cause irritation or burns to the skin and eyes. If contact occurs, flush immediately with water.
8. Do not interchange components from kits with different batch numbers. The components have been standardized as a unit for a given batch.
9. Hemolyzed, hyperlipemic, heat-treated or contaminated specimens may give erroneous results.
10. Do not dilute serum specimens directly in the microwells.
11. Do not touch or scrape the bottom of the microwells when pipetting or aspirating fluid.
12. Incubation times and temperatures other than those specified may give erroneous results.
13. Do not allow the wells to dry once the assay has begun.
14. Keep the TMB Substrate away from bright light.
15. Do not reuse microwells or pour reagents back into their bottles once dispensed.

### PRECAUTIONS

#### For *in vitro* diagnostic use only

1. The properdin calibrators were prepared from pooled human sera. Each blood unit used for its preparation was tested by approved methods and found to be nonreactive for hepatitis B surface antigen (HBsAg) and antibodies against human immunodeficiency virus (HIV) 1 and 2, and hepatitis C virus (HCV). However, as no test method can offer complete security that infectious agents are absent, the calibrators and patients' specimens should be handled at Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the CDC/NIH manual "Biosafety In Microbiology and Biomedical Laboratories", 1999. Solutions containing human serum should be treated as potentially infectious and handled accordingly.
2. Use separate pipette tips for each sample, calibrator and reagent to avoid cross-contamination.
3. Use separate reservoirs for each reagent. This applies especially to the TMB Substrate.
4. After use, decontaminate all specimens, reagents and materials by soaking for at least 30 minutes in sodium hypochlorite solution (household bleach diluted 1:10).
5. To avoid droplet formation during washing, aspirate the wash solution into a bottle containing bleach.
6. Reagents in this kit are preserved with up to 0.375% thimerosal, also called thiomersal or merthiolate, corresponding to 0.015% in the final

### STABILITY AND STORAGE

1. Store the kit with all reagents at 2-8°C. Do not freeze.
2. Use all reagents before the expiry date on the vial labels.
3. Diluted Wash Solution remains stable for 3 months at 2-8°C. If not using all strips, dilute only the portion of Wash Solution Concentrate required.
4. For subsequent use, store unused well strips in the foil pouch with the desiccant provided and reseal.

### COLLECTION OF SPECIMENS

**Handle and dispose of all blood or serum specimens as if they were potentially infectious. See Precautions, sections 1, 2, 4 and 5.**

Determination of properdin in a single specimen requires 10 µL of serum or plasma. Blood specimens should be collected aseptically into plain, heparinized or EDTA tubes by qualified staff using approved venepuncture techniques. Serum or plasma should be prepared by standard techniques for clinical laboratory testing. Cap the serum or plasma specimens and freeze them at -20°C or below. This especially applies if the assay cannot be performed within 24 hours or if the specimen is to be shipped. For long-term storage of serum or plasma, -70°C or below is recommended. **Avoid repeated freezing and thawing.** Do not use hemolyzed, hyperlipemic, heat-treated or contaminated specimens.

### PREPARATION OF REAGENTS AND SAMPLES

1. Bring all specimens and reagents to room temperature (20-25°C). Mix specimens thoroughly

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- by gentle inversion and if necessary clear visible particulate matter by low-speed centrifugation.
- Determine the number of specimens to be tested (in duplicate) plus any internal laboratory control specimens (in duplicate) plus any reagent blank wells. Add 16 wells for the 8 calibrators (in duplicate). Remove the number of microwell strips required and replace the remainder in the foil pouch with desiccant at 2-8°C.
  - Wash Solution: Dilute the 25X Wash Solution Concentrate by pouring the total contents of the bottle (30 mL) into a 1-L graduated cylinder and add distilled or deionized water to a final volume of 750 mL. Mix thoroughly and store at 2-8°C. If not using all strips, dilute only the portion of Wash Solution Concentrate required.
  - Sample Diluent: ready to use, do not dilute further.
  - Properdin Calibrators: ready to use. The assigned concentrations are indicated on their labels. Do not dilute further.
  - Biotinylated Properdin Antibody: ready to use, do not dilute further.
  - HRP-Streptavidin Conjugate: ready to use, do not dilute further.
  - TMB Substrate: ready to use, do not dilute further.
  - Stop Solution: ready to use, do not dilute further.
  - Patient specimens: Dilute each specimen in a recorded proportion with Sample Diluent to obtain at least 250 µL of diluted solution that can be set up in duplicate wells at 100 µL per well. An initial screening at a dilution of 1/500 is recommended. This can be prepared in two steps, as follows: dilute 10 µL of serum in 190 µL of Sample Diluent to make a 1/20 dilution; then dilute 10 µL of the 1/20 dilution in 240 µL of Sample Diluent to make a 1/500 dilution. Dilutions are mixed by inversion or moderate vortexing. Reassay of out-of-range samples at lower or higher dilution is rarely necessary. Dilutions lower than 1/10 should not be used.
- at room temperature on a shaking platform set at 200/minute.
- Aspirate the contents of the microwells and wash the microwells three times with 300 µL diluted Wash Solution. If washing is done manually, empty the microwells by inversion and gentle shaking into a suitable container, followed by blotting in the inverted position on a paper towel. A dwell time of 1 minute before emptying is recommended for at least the last wash of the cycle.
  - Dispense 100 µL of Biotinylated Properdin Antibody (ready to use) into each microwell. A multichannel or repeating micropipette can be used. Cover the wells and incubate for 60 minutes at room temperature on a shaking platform (200/minute).
  - Wash as described above in Step 3.
  - Dispense 100 µL of HRP-Streptavidin Conjugate (ready to use) into each microwell. A multichannel or repeating micropipette can be used. Cover the wells and incubate for 60 minutes at room temperature on a shaking platform (200/minute).
  - Wash as described above in Step 3.
  - Dispense 100 µL of TMB Substrate (ready to use) into each microwell. The use of a multichannel micropipette is recommended to reduce pipetting time. Cover the wells and incubate for **exactly 15 minutes** at room temperature in the dark. Start the clock when filling the first well.
  - Add 100 µL Stop Solution (ready to use) to each well, maintaining the same pipetting sequence and rate as in Step 8. Mix by gentle shaking for 20 seconds, avoiding splashing. Read the wells within 30 minutes.
  - Read the optical densities (absorbances) of the wells at 450 nm in an appropriate microplate reader (reference wave-length 650 or 620 nm). If no reference wavelength is available, the value of the reagent blank well is subtracted from each of the other values before other calculations are performed.

### ASSAY PROCEDURE

(See also schematic overview on next page)

- Prepare the assay protocol, assigning the appropriate wells for setting up calibrators, diluted patient specimens and any internal laboratory controls in duplicate. If a reference wavelength of 650 or 620 nm is not available on the ELISA reader, a reagent blank well can be assigned. This is set up with 100 µL of Sample Diluent instead of diluted serum and processed like the other wells.
- Pipette 100 µL volumes of each calibrator, diluted specimens and any internal laboratory controls into the corresponding positions in the microwell strips. Cover the wells and incubate for 60 minutes

### CALCULATION OF RESULTS

The basic principle is to construct a calibration curve by plotting the mean of duplicate optical density values for each Properdin Calibrator on the y-axis against the corresponding properdin concentrations in ng/mL on the x-axis. The calibration curve must meet the validation requirements. The properdin concentration of each diluted serum sample is then found by locating the point on the curve corresponding to the mean of duplicate optical density values for the diluted serum sample and reading its corresponding concentration in mU/mL from the x axis. The concentration of properdin in the

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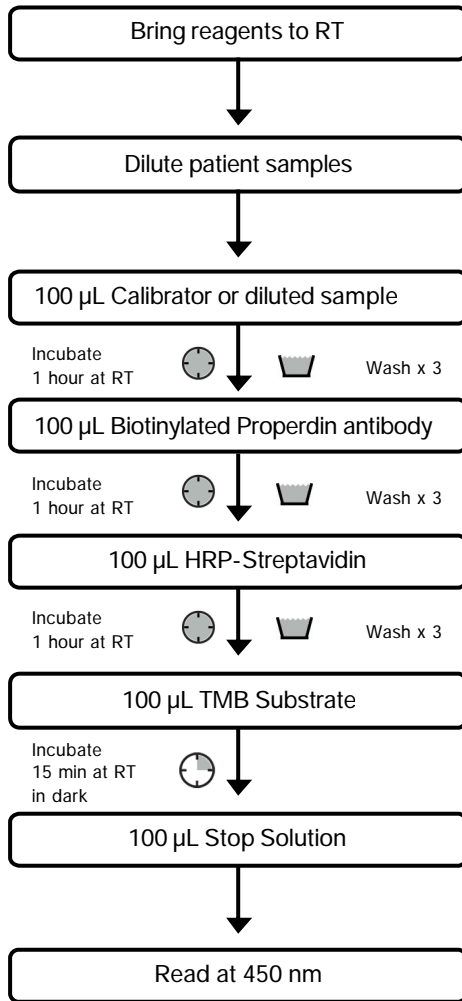
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undiluted sample is calculated by multiplying this result by the sample dilution factor.

### Schematic overview of assay procedure



### CALCULATION OF RESULTS (contd.)

This procedure can be performed manually using graph paper with linear x and y scales. A smooth curve can be drawn through the points or adjacent points can be joined by straight lines. The latter procedure may slightly overestimate/underestimate concentration values between points when the curve is slightly convex to left/right, respectively. Although the curve may approximate to a straight line, it is both practically and theoretically incorrect to calculate and draw the straight line of best fit and to read the results from this.

The procedure can also be performed by an ELISA reader software program incorporating curve fitting

procedures. The procedure of choice is to use linear x and y axes with 4-parameter logistic curve fitting. Diluted samples that give a mean optical density value above that for the 500 mU/mL Properdin Calibrator or below that for the 5 mU/mL Properdin Calibrator are out of the range of the assay and their concentrations should be noted as >500 mU/mL and <5 mU/mL respectively. The corresponding concentrations in the undiluted sera are calculated >(500 x dilution factor) mU/mL and <(5 x dilution factor) mU/mL, respectively. If necessary, these samples can be re-assayed at higher and lower dilutions for high- and low-reading samples, respectively. The new dilution factors should be those estimated to give optical density values that fall well within the range of the calibration curve, but dilutions lower than 1/10 should not be used.

### VALIDATION OF CALIBRATION CURVE

The mean optical density values for the 500 mU/mL and 0 mU/mL Properdin Calibrators should be >1.5 and <0.05, respectively. Any curvature should be slight when the results are plotted on linear axes.

**Out-of-line points for individual calibrators:** One or more individual calibrators may give anomalous OD readings. One or both of the duplicate values may be out of line, and the mean of the duplicates may be out of line. This error is significant if it impairs satisfactory curve fitting by the 4-parameter logistic method, which, as a result of the anomalous value, is shifted away from other calibrator points that are in fact correct. The calibrator points and fitted curve should always be examined for correct fit before any calculations of concentration from it are accepted. A poorly fitting curve will also be revealed by a high  $R^2$  value. If only one calibrator is affected, which is not the highest calibrator, two courses of action are possible:

i) An erroneous singlet or duplicate result should be eliminated from the curve, and the remaining results refitted by the 4-parameter logistic procedure. If a satisfactory fit is obtained, provisional concentration results can be calculated from it.

ii) If no satisfactory fit can be obtained in this way, but the curve is otherwise consistent, provisional results can be obtained from straight lines or simple cubic spline fitting between the means of duplicates, omitting the erroneous point.

If two or more calibrators are affected, the assay should be repeated.

A deviant result for an individual calibrator can be due to operator error or to calibrator deterioration. If both duplicate values are consistently out of line in successive assays, the calibrator is faulty and should be omitted.

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### INTERPRETATION OF RESULTS

The full range of properdin concentrations in serum or plasma from healthy human donors as measured by this assay is 60-171 U/mL. For want of an international properdin standard, the WHO international reference preparation W1032 for human complement components C1q, C4, C5, factor B and whole functional complement CH50 was assigned a value for properdin of 100 U/mL and the calibrator material was standardized against this preparation. As the reference preparation is derived from normal human serum, the properdin concentrations in serum or plasma from healthy donors are expected to be in the region of 100 U/mL, and samples diluted 1/500 are expected to show concentrations in the region of 200 mU/mL. Assay of a preparation of purified properdin against the reference preparation showed that 100 U was equivalent to 18.7 µg of properdin. If it is preferred to record results as µg of properdin per mL, the value in U/mL must be multiplied by 0.187 to give the value in µg/mL.

Type-1 properdin deficiency is characterized by serum or plasma concentrations <0.1 µg/mL (<0.5 U/mL); type-2 properdin deficiency by serum or plasma concentrations of about 2 µg/mL (about 10 U/mL); type-3 (functional) properdin deficiency will show normal properdin concentrations and will not be detected by this assay. Female carriers will show properdin concentrations between normal and deficient values.

### QUALITY CONTROL

Laboratories intending to perform repeated assays should establish their own high-reading (>120 U/mL) and low-reading (<60 mU/mL) control sera, stored in small (e.g. 50-µL) aliquots at -70°C or below. An aliquot of each should be thawed and tested in each assay and a record kept of successive results. This serves as a control of test performance, test integrity and operator reliability. The results should be examined for drift (tendency for successive results to rise or fall) or significant deviation from the mean of previous results. Values not deviating by more than 20% from the mean of previous values can be taken to indicate acceptability of the assay. Aliquots of control serum should not be refrozen for repeated assay once thawed, and if a further assay is performed, fresh control aliquots and fresh dilutions of patient specimens should be used.

### LIMITATIONS

An abnormal high or low concentration of properdin in serum does not necessarily imply the existence of any disease. Physicians must interpret the significance of the properdin value in the light of the clinical features.

Special care should be taken when handling the samples to be measured, as properdin immuno-reactivity is greatly influenced by storage conditions (see below). Samples should be frozen as quickly as possible and should not be thawed until directly prior to assay.

### EXPECTED RESULTS

23 serum specimens from healthy Danish and American blood donors were assayed in the ANTIBODYSHOP Properdin ELISA Kit. Properdin concentrations ranged from 60 U/mL to 171 U/mL. Mean and median concentrations were 122 U/mL and 123 U/mL, respectively, with a standard deviation (SD) of 24.7 U/mL. These samples were frozen and thawed 1-2 times prior to assay.

15 aged plasma samples (from healthy Danish donors) that been stored at -20°C and thawed and refrozen an unknown number of times over a period of 10 years, showed properdin concentrations from 74 U/mL to 141 U/mL. Mean and median concentrations were 102 U/mL and 100 U/mL, respectively, with an SD of 20.1 U/mL.

6 serum specimens from Danish males with known type-1 properdin deficiency showed properdin concentrations of <0.5 U/mL, and samples from 9 female carriers gave values between 28 and 59 U/mL.

### PERFORMANCE CHARACTERISTICS

**Limit of detection:** The lowest concentration of properdin giving an OD reading greater than 2 SD above the mean zero calibrator reading (n = 6) was 0.37 mU/mL, corresponding to a serum concentration of 0.2 U/mL in a specimen diluted 1/500.

**Intraassay (within-run) and interassay (between-run, different days/operators) reproducibility:** Three individual serum samples and one serum pool were run in 6 replicates in 4 separate assays performed by 3 different operators to determine both within-run and between-run reproducibility.

The following results were obtained (CV = coefficient of variation):

Parameter	CV acceptance limit	CV of current batch
Intraassay	≤20%	4.6-16.9%
Interassay	≤25%	3.3-18.9%

**Sample material:** The properdin concentration in EDTA and heparin plasma was measured and expressed as a percentage of the value in serum from the same bleed by 4 different operators.

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Sample	#1	#2	#3	#4	Mean
Serum	100%	100%	100%	100%	100%
EDTA plasma	106.8%	91.1%	104.7%	107.3%	102.5%
Heparin plasma	103.1%	91.2%	96.4%	101.0%	97.9%

**Linearity:** A serum pool with high content of properdin (152 U/mL) was assayed over a wide range of dilutions (1/310 – 1/25000). The values obtained are given as a percentage of the theoretical value.

Dilution	310	760	1500	3000	7600	15200	25000
Recovery	91%	100%	100%	99%	98%	93%	111%

**Specificity:** The specificity of the monoclonal antibody has been verified by demonstrating binding to purified properdin in combination with readings indistinguishable from zero obtained from diagnosed properdin-deficient donors.

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### LIABILITY

This kit is only intended for the *in vitro* determination of properdin in human serum or plasma.

The kit is only intended for use by qualified personnel carrying out research or diagnostic activities.

If the recipient of this kit passes it on in any way to a third party, this instruction must be enclosed, and said recipient shall at own risk secure in favor of ANTIBODYSHOP A/S all limitations of liability herein.

ANTIBODYSHOP A/S shall not be responsible for any damages or losses due to using the kit in any way other than as expressly stated in these Instructions.

The liability of ANTIBODYSHOP A/S shall in no event exceed the commercial value of the kit.

ANTIBODYSHOP A/S shall under no circumstances be liable for indirect, special or consequential damages, including but not limited to loss of profit.



**For *in vitro* diagnostic use only**

The Properdin ELISA Kit conforms to Directive 98/79/EU of the European Parliament and Council of 27 October 1998 on *in vitro* diagnostic medical devices. FDA approval for diagnostic use in the USA has not been sought.

