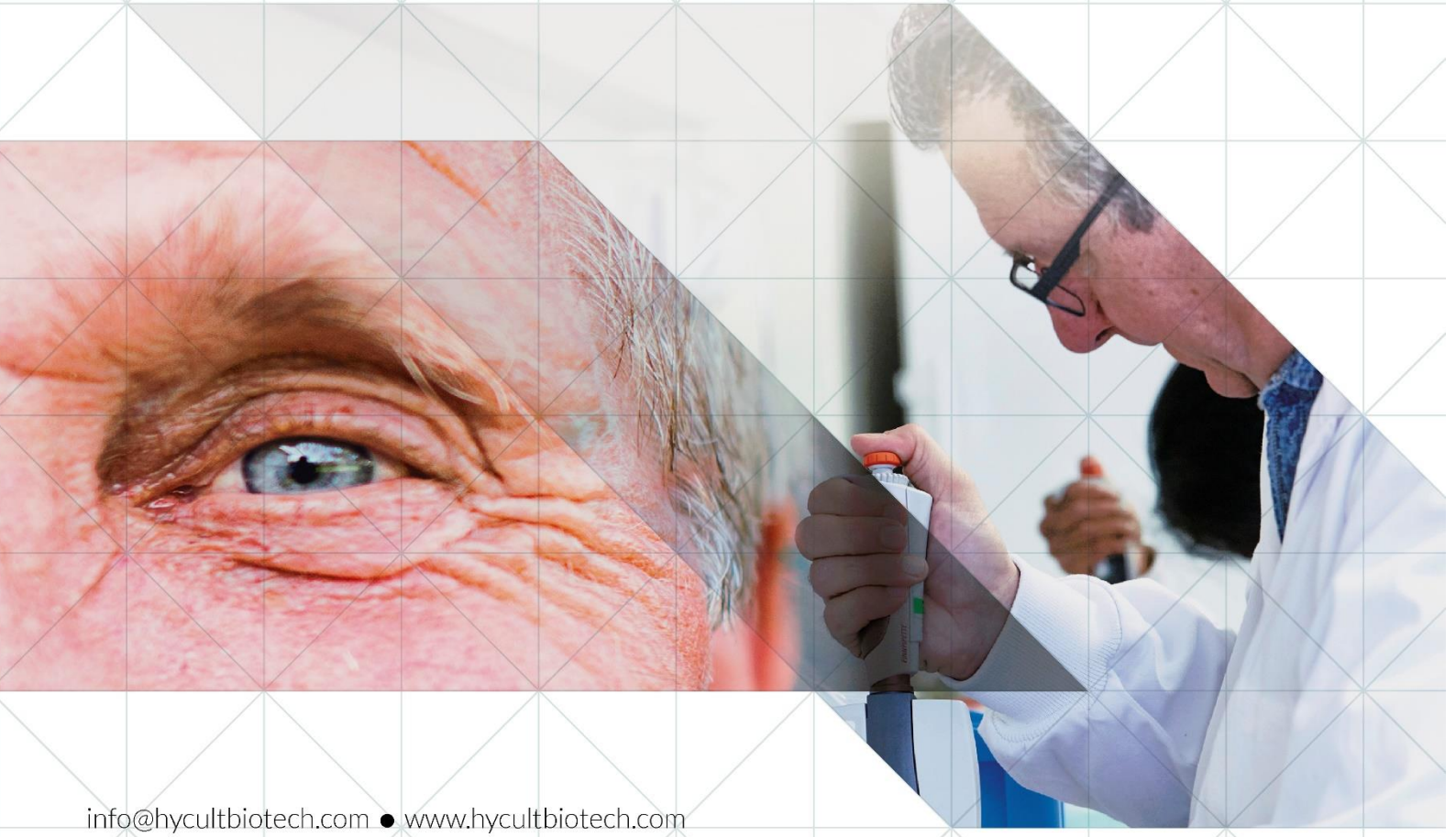
# Human Complement Factor H

## HK342

**Edition 04-17**

**ELISA KIT**
**PRODUCT INFORMATION & MANUAL**

Read carefully prior to starting procedures!
For use in laboratory research only
Not for clinical or diagnostic use



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Note that this user protocol is not lot-specific and is representative for the current specifications of this product. Please consult the vial label and the Certificate of Analysis for information on specific lots. Also note that shipping conditions may differ from storage conditions.

For research use only. Not for use in or on humans or animals or for diagnostics. It is the responsibility of the user to comply with all local/state and federal rules in the use of this product. Hycult Biotech is not responsible for any patent infringements that might result from the use or derivation of this product.

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## 1. INTENDED USE

The human complement factor H ELISA kit is to be used for the *in vitro* quantitative determination of human complement factor H in serum, plasma and urine samples. This kit is intended for laboratory research use only and is not for use in diagnostic or therapeutic procedures.

The analysis should be performed by trained laboratory professionals.

## 2. INTRODUCTION

The complement system mediates a number of essential biological functions that participate in host defense against infection, initiation of the inflammatory reaction, processing and clearance of immune complexes and regulation of the immune response.

There are three pathways of complement activation. The classical pathway is initiated by immune complexes; the lectin pathway by surface bound mannan binding lectin; and the alternative pathway by all the surfaces that are not specifically protected against it. Each generates a C3 convertase, a serine protease that cleaves the central complement protein C3, and generates the major cleavage fragment C3b.

Complement factor H is a relatively abundant plasma protein, with a concentration of 400-800 µg/ml, that is essential to maintain complement homeostasis and to restrict the action of complement to activating surfaces. Factor H binds to C3b, accerates the decay of the alternative pathway C3-convertase (C3bBb) and act as co-factor for the factor I-mediated proteolytic inactivation of C3b. Factor H regulates complement both in fluid phase and on cellular surfaces.

Complement protein factor H is the first regulatory protein of the alternative pathway. Factor H is a single-chain serum glycoprotein of 150 kDa with a modular structure consisting of a tandem of 20 homologous units of about 60 amino acid, called short consensus repeats (SCR).

Numerous functional sites have been identified along the 20 SCR domain structure of factor H. Three C3-binding sites have been identified; in the SCR1-4 in SCR6-10 and SCR13-20. Three polyanion binding sites like heparin and several glycoaminoglycans have also been identified in the SCR7, 13 and 20. Factor H displays anti-inflammatory functions and acts as a ligand for CRP.

Factor H has two important functional domains that are located at the opposite ends of the protein. The N-terminal fragment of the factor H molecule is an essential fluid phase regulator of the alternative pathway. With the C terminal domain and SCR 7 factor H binds to cell and tissue surface and thus mediates its protective role also on host cell surface.

Genetic analyses reveal a clear association of complement factor H with different human diseases. These incude diseases of the kidney, the atypical form of Hemolytic Uremic Syndrome (aHUS) and membraneoproliferative glomerulonephritis (MPGN), and of the eye, age-related macular degeneration (AMD).

## 3. KIT FEATURES

- Working time of 2½ hours.
- Minimum concentration which can be measured is 3.9 ng/ml.
- Measurable concentration range of 3.9 to 250 ng/ml.
- Working volume of 100 µl/well.

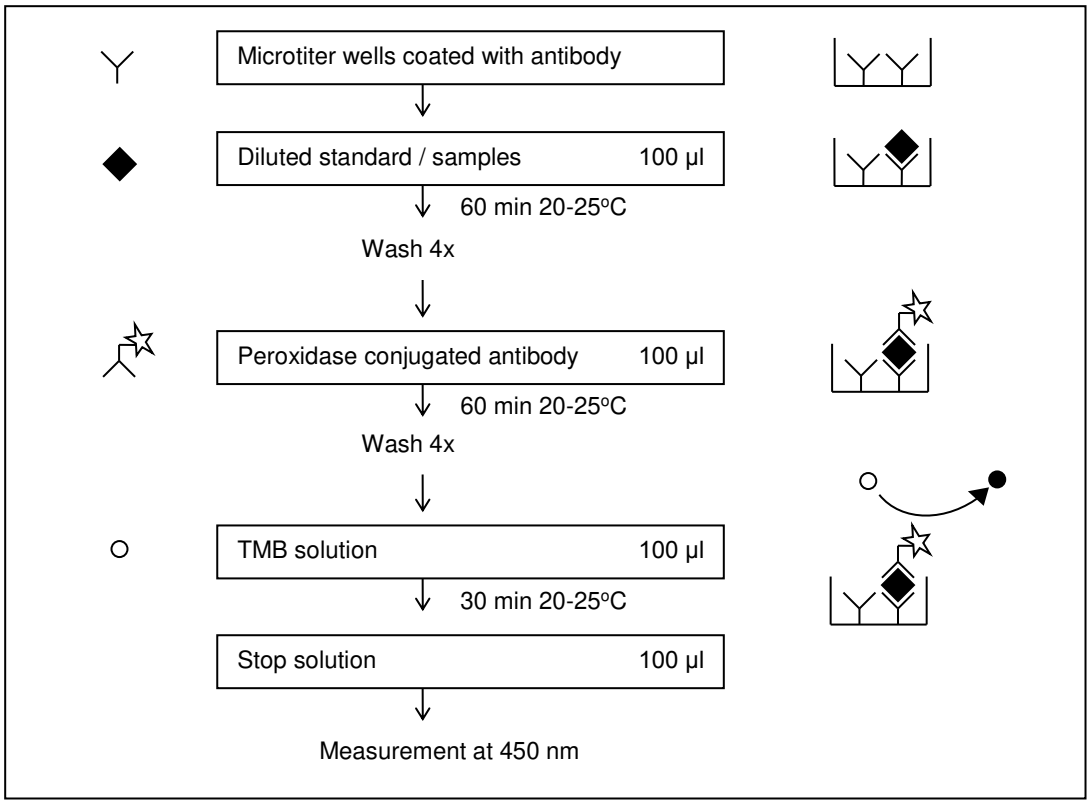
## Cross reactivity

| Cross reactant | Reactivity |
|---|---|
| Bovine Complement Factor H | negative |
| Horse Complement Factor H | negative |
| Mouse Complement Factor H | negative |
| Rat Complement Factor H | negative |
| CFHR1, recombinant | negative |
| CFHR3, recombinant | negative |
| CFHR4, recombinant | negative |
| CFHR5, recombinant | negative |
| HFL3, recombinant | negative |
| HF-1, recombinant | negative |

Table 1

Cross-reactivity for other species or proteins/peptides has not been tested.

## 4. PROTOCOL OVERVIEW




- The human complement factor H ELISA is a ready-to-use solid-phase enzyme-linked immunosorbent assay based on the sandwich principle with a working time of 2½ hours.
- The efficient format of a plate with twelve disposable 8-well strips allows free choice of batch size for the assay.
- Samples and standards are incubated in microtiter wells coated with antibodies recognizing human complement factor H.
- Peroxidase conjugate antibody will bind to the captured human complement factor H.
- Peroxidase conjugate antibody will react with the substrate, tetramethylbenzidine (TMB).
- The enzyme reaction is stopped by the addition of oxalic acid.
- The absorbance at 450 nm is measured with a spectrophotometer. A standard curve is obtained by plotting the absorbance (linear) versus the corresponding concentrations of the human complement factor H standards (log).
- The human complement factor H concentration of samples, which are run concurrently with the standards, can be determined from the standard curve.

## 5. KIT COMPONENTS AND STORAGE INSTRUCTIONS

| Kit component | Cat.# | Quantity HK342-01 | Quantity HK342-02 | Color code |
|---|---|---|---|---|
| Wash buffer 20x | WB21 | 1 vial (60 ml) | 1 vial (60 ml) | Colorless |
| Dilution buffer 10x | DB89 | 1 vial (20 ml) | 1 vial (20 ml) | Green |
| Standard | | 2 vials, lyophilized | 4 vials, lyophilized | White |
| Conjugate, peroxidase-labeled | | 1 vial, 1 ml lyophilized | 2 vials, 1 ml lyophilized | Blue |
| TMB substrate | TMB050/TMB100 | 1 vial (11 ml) | 1 vial (22 ml) | Brown |
| Stop solution | STOP110 | 1 vial (22 ml) | 1 vial (22 ml) | Red |
| 12 Microtiter strips, pre-coated | | 1 plate | 2 plates | |
| Certificate of Analysis | | 1 | 1 | |
| Manual | | 1 | 1 | |
| Data collection sheet | | 2 | 2 | |

Table 2

- Upon receipt, store individual components at 2 - 8°C. Do not freeze.
- Do not use components beyond the expiration date printed on the kit label.
- The standard and conjugate are stable in lyophilized form until the expiration date indicated on the kit label, if stored at 2 - 8°C.
- The exact amount of the standard is indicated on the label of the vial and the Certificate of Analysis.
- After reconstitution the standard must be used within 1 hour. The standard can not be stored for repeated use.
- Once reconstituted conjugate is stable for 1 month if stored at 2 - 8°C.
- Upon receipt, foil pouch around the plate should be vacuum-sealed and unpunctured. Any irregularities to aforementioned conditions may influence plate performance in the assay.
- Return unused strips immediately to the foil pouch containing the desiccant pack and reseal along the entire edge of the zip-seal. Quality guaranteed for 1 month if stored at 2 - 8°C.

**Materials requires but not provided**

- Calibrated micropipettes and disposable tips.
- Distilled or de-ionized water.
- Plate washer: automatic or manual.
- Polypropylene tubes.
- Calibrated ELISA plate reader capable of measuring absorbance at 450 nm.
- Adhesive covers can be ordered separately. Please contact your local distributor.

## 6. WARNINGS AND PRECAUTIONS

- For research use only, not for diagnostic or therapeutic use.
- This kit should only be used by qualified laboratory staff.
- Do not under any circumstances add sodium azide as preservative to any of the components.
- Do not use kit components beyond the expiration date.
- Do not mix reagents from different kits and lots. The reagents have been standardized as a unit for a given lot. Use only the reagents supplied by the manufacturer.
- The assay has been optimized for the indicated standard range. Do not change the standard range.
- Open vials carefully: vials are under vacuum.
- Do not ingest any of the kit components.
- Kit reagents contain 2-chloroacetamide as a preservative. 2-Chloroacetamide is harmful in contact with skin and toxic if swallowed. In case of accident or if you feel unwell, seek medical advice immediately.
- The TMB substrate is light sensitive, keep away from bright light. The solution should be colourless until use.
- The stop solution contains 2% oxalic acid and can cause irritation or burns to respiratory system, skin and eyes. Direct contact with skin and eyes should be strictly avoided. If contact occurs, rinse immediately with plenty of water and seek medical advice.
- Incubation times, incubation temperature and pipetting volumes other than those specified may give erroneous results.
- Do not reuse microwells or pour reagents back into their bottles once dispensed.
- Handle all biological samples as potentially hazardous and capable of transmitting diseases.
- Hemolyzed, hyperlipemic, heat-treated or contaminated samples may give erroneous results.
- Use polypropylene tubes for preparation of standard and samples. Do not use polystyrene tubes or sample plates.
- The standard is of human origin. It was tested for various viruses and found negative. Since no test method can offer complete assurance that infectious agents are absent, this reagent should be handled as any potentially infectious human serum or blood specimen. Handle all materials in contact with this reagent according to guide-lines for prevention of transmission of blood-borne infections.

## 7. SAMPLE PREPARATION

### Collection and handling

#### Serum or plasma

Collect blood using normal aseptic techniques. Blood samples should be kept on ice. If serum is used, separate serum from blood after clotting within 20 minutes by centrifugation (1,500xg at 4°C for 15 min). Transfer the serum to a fresh polypropylene tube.

If plasma is used, separate plasma from blood within 20 minutes after blood sampling by centrifugation (1,500xg at 4°C for 15 min). Transfer the plasma to a fresh polypropylene tube.

Most reliable results are obtained if EDTA plasma is used.

#### Urine

Collect urine using normal aseptic techniques. Centrifuge the urine to remove debris (1500xg at 4 °C for 15 min). Transfer urine to a fresh polypropylene tube.

#### Storage

Store samples below -20°C, preferably at -70°C in polypropylene tubes. Storage at -20°C can affect recovery of human complement factor H. Use samples within 24 hours after thawing. Avoid multiple freeze-thaw cycles which may cause loss of human complement factor H activity and give erroneous results.

Do not use hemolyzed, hyperlipemic, heat-treated or contaminated samples.

Before performing the assay, samples should be brought to room temperature (18 – 25°C) and mixed gently. Prepare all samples (controls and test samples) prior to starting the assay procedure. Avoid foaming.

### Dilution procedures

#### Serum or plasma samples

Human complement factor H can be measured accurately if serum or plasma samples are diluted at least 5,000x with supplied dilution buffer in polypropylene tubes.

Note that most reliable results are obtained with EDTA plasma.

#### Urine samples

Human complement factor H can be measured accurately if urine samples are diluted at least 4x with supplied dilution buffer in polypropylene tubes.

#### Comment regarding recommended sample dilution

The mentioned dilution for samples is a minimum dilution and should be used as a guideline. The recovery of human complement factor H from an undiluted sample is not 100% and may vary from sample to sample. When testing less diluted samples it is advisable to run recovery experiments to determine the influence of the matrix on the detection of human complement factor H.

Do not use polystyrene tubes or sample plates for preparation or dilution of the samples.

## 8. REAGENT PREPARATION

Allow all the reagents to equilibrate to room temperature (20 – 25°C) prior to use. Return to proper storage conditions immediately after use.

### Wash buffer

Prepare wash buffer by mixing 60 ml of 20x wash buffer with 1140 ml of distilled or de-ionized water, which is sufficient for 2 x 96 tests. Where less volume is required, prepare the desired volume of wash buffer by diluting 1 part of the 20x wash buffer with 19 parts of distilled or de-ionized water.

### Dilution buffer

Prepare dilution buffer by mixing 20 ml of the 10x dilution buffer with 180 ml of distilled or de-ionized water, which is sufficient for 2 x 96 tests. Where less volume is required, prepare the desired volume of dilution buffer by diluting 1 part of the 10x dilution buffer with 9 parts of distilled or de-ionized water. Concentrated dilution buffer may contain crystals. In case the crystals do not disappear at room temperature within 1 hour, concentrated dilution buffer can be warmed up to 37°C. Do not shake the solution.

### Standard solution

The standard is reconstituted with the amount of dilution buffer mentioned on the CoA in the standard vial. Use the standard vial as Tube 1 in Figure 1.

The standard can not be stored for repeated use. Prepare each human complement factor H standard in polypropylene tubes by serial dilution of the reconstituted standard with dilution buffer as shown in Figure 1*. The standard cannot be stored for repeated use.

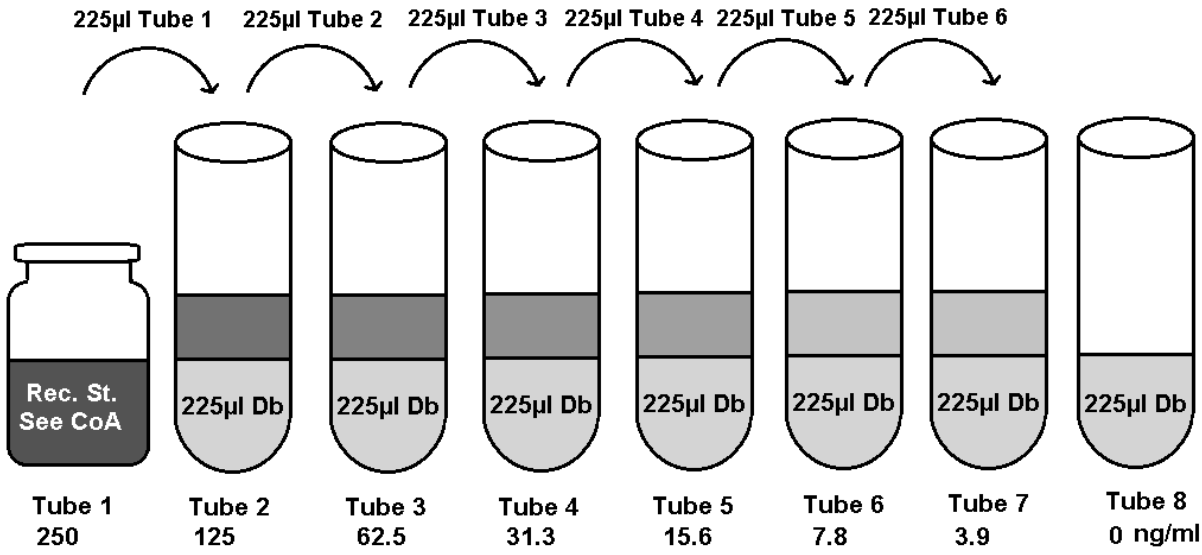



Figure 1

*) CoA: Certificate of Analysis, Rec. St: Reconstituted Standard, Db: Dilution buffer

### Conjugate solution

The conjugate is reconstituted by pipetting 1 ml distilled or de-ionized water. Dilute the reconstituted 1 ml conjugate with 11 ml dilution buffer, which is sufficient for 1 x 96 tests. In case less volume is required, prepare the desired volume of conjugate by diluting 1 part of the reconstituted conjugate with 11 parts of dilution buffer.

## 9. ELISA PROTOCOL

Bring all reagents to room temperature (20 - 25°C) before use.

1. Determine the number of test wells required, put the necessary microwell strips into the supplied frame, and fill out the data collection sheet. Return the unused strips to the storage bag with desiccant, seal and store at 2 - 8°C.
2. Transfer 100 µl in duplicate of standard, samples, or controls into appropriate wells. Do not touch the side or bottom of the wells.
3. Cover the tray and tap the tray to eliminate any air bubbles. Be careful not to splash liquid onto the cover.
4. Incubate the strips or plate for 1 hour at room temperature.
5. Wash the plates 4 times with wash buffer as follows*:
   a. Carefully remove the cover, avoid splashing.
   b. Empty the plate by inverting plate and shaking contents out over the sink, keep inverted and tap dry on a thick layer of tissues.
   c. Add 200 µl of wash buffer to each well, wait 20 seconds, empty the plate as described in 5b.
   d. Repeat the washing procedure 5b/5c three times.
   e. Empty the plate and gently tap on thick layer of tissues.
6. Add 100 µl of diluted conjugate to each well using the same pipetting order as applied in step 2. Do not touch the side or bottom of the wells.
7. Cover the tray and incubate the tray for 1 hour at room temperature.
8. Repeat the wash procedure described in step 5.
9. Add 100 µl of TMB substrate to each well, using the same pipetting order as applied in step 2. Do not touch the side or bottom of the wells.
10. Cover the tray and incubate the tray for 30 minutes at room temperature. It is advised to control the plate regularly. In case of strong development the TMB reaction can be stopped sooner. Avoid exposing the microwell strips to direct sunlight. Covering the plate with aluminium foil is recommended.
11. Stop the reaction by adding 100 µl of stop solution with the same sequence and timing as used in step 12. Mix solutions in the wells thoroughly by gently swirling the plate. Gently tap the tray to eliminate any air bubbles trapped in the wells.
12. Read the plate within 30 minutes after addition of stop solution at 450 nm using a plate reader, following the instructions provided by the instrument's manufacturer.

*) In case plate washer is used, please note: use of a plate washer can result in higher background and ` decrease in sensitivity. We advise validation of the plate washer with the manual procedure. Make sure the plate washer is used as specified for the manual method.

## 10. INTERPRETATION OF RESULTS

- Calculate the mean absorbance for each set of duplicate standards, control and samples.
- If individual absorbance values differ by more than 15% from the corresponding mean value, the result is considered suspect and the sample should be retested.
- The mean absorbance of the zero standard should be less than 0.3.
- Create a standard curve using computer software capable of generating a good curve fit. The mean absorbance for each standard concentration is plotted on the vertical (Y) axis versus the corresponding concentration on the horizontal (X) axis (logarithmic scale).
- If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
- Samples that give a mean absorbance above the absorbance for the highest standard concentration are out of range of the assay. These samples should be retested at a higher dilution.

## 11. TECHNICAL HINTS

- User should be trained and familiar with ELISA assays and test procedure.
- If user is not familiar with the ELISA technique it is recommended that the user perform a pilot assay prior to evaluation of your samples. Perform the assay with a standard curve only following the instructions.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Completely empty wells before dispensing wash buffer, fill with wash buffer as indicated for each cycle and do not allow wells to sit uncovered or dry for extended periods.
- Since exact conditions may vary from assay to assay, a standard curve must be established for every run. Samples should be referred to the standard curve prepared on the same plate.
- Do not mix reagents from different batches, or other reagents and strips. Remainders should not be mixed with contents of freshly opened vials.
- Each time the kit is used, fresh dilutions of standard, sample, conjugate and buffers should be made.
- Caps and vials are not interchangeable. Caps should be replaced on the corresponding vials.
- To avoid cross-contaminations, change pipette tips between reagent additions of each standard, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- Waste disposal should be performed according to your laboratory regulations.

**Technical support**

Do not hesitate to contact our technical support team at support@hycultbiotech.com for inquiries and technical support regarding the human Factor H ELISA.

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## 12. QUALITY CONTROL

The Certificate of Analysis included in this kit is lot specific and is to be used to verify results obtained by your laboratory. The absorption values provided on the Certificate of Analysis are to be used as a guideline only. The results obtained by your laboratory may differ.

This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

For optimal performance of this kit, it is advised to work according to good laboratory practice.

## 13. PERFORMANCE CHARACTERISTICS

### Adaptation of standard using Dr. Opperman values converted to Dr. Morgan values

Six EDTA plasma donors were tested using the Opperman standard (3.9 – 250 ng/ml) or the Morgan standard (0.9 – 59.6 ng/ml). The resulting CFH values of the 6 donor EDTA samples are expressed in Opperman and in Morgan standard mg/ml values .

| EDTA plasma donor | [CFH] mg/ml Opperman | [CFH] mg/ml Morgan |
|---|---|---|
| M/66427 | 0.73 | 0.17 |
| M/66102 | 1.17 | 0.28 |
| F/45929 | 1.22 | 0.29 |
| M/68211 | 0.57 | 0.14 |
| M/67034 | 0.26 | 0.06 |
| M/60818 | 0.71 | 0.18 |

### Recovery

Normal human blood samples (plasma), containing baseline levels of human complement factor H, were spiked with human complement factor H (CFH) in concentrations of 50 and 100 ng/ml. Samples with and without human complement factor H were incubated for 1 hour at room temperature. Samples were measured using the ELISA. Values for human complement factor H ranged between 96% and 108% (mean 102%).

## 14. TROUBLESHOOTING

Warranty claims and complaints in respect of deficiencies must be logged before expiry date of the product. A written complaint containing lot number of the product and experimental data shall be sent to support@hycultbiotech.com. Suggestions summarized below in Table 3 can be used as guideline in case of unexpected assay results.

| Low absorbance | High absorbance | Poor duplicates | All wells positive | All wells negative | Possible cause |
|---|---|---|---|---|---|
| • | • | | • | • | Kit materials or reagents are contaminated or expired |
| • | | | | | Incorrect reagents used |
| • | | • | • | | Lyophilized reagents are not properly reconstituted |
| • | • | • | • | • | Incorrect dilutions or pipetting errors |
| • | | • | | | Improper plastics used for preparation of standard and/or samples |
| • | • | | | | Improper incubation times or temperature |
| | | • | | | Especially in case of 37°C incubation: plates are not incubated uniformly |
| • | | | | | Assay performed before reagents had reached room temperature |
| • | • | • | • | • | Procedure not followed correctly |
| | | | | • | Omission of a reagent or a step |
| | | • | | | Poor mixing of samples |
| | • | | • | | Low purity of water |
| | • | • | | | Strips were kept dry for too long during/after washing |
| | • | • | • | | Inefficient washing |
| | • | • | | | Cross-contamination from other samples or positive control |
| | | • | • | | TMB solution is not clear or colourless |
| • | • | | | | Wrong filter in the microtiter reader |
| | • | • | | | Airbubbles |
| | | • | | | Imprecise sealing of the plate after use |
| • | | | | | Wrong storage conditions |
| • | | | | | Lamp in microplate reader is not functioning optimally |

Table 3

## 15. REFERENCES


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