



**EAGLE**  
BIOSCIENCES

# **Complement Factor H-Related Protein 3 (FHR-3) ELISA Kit**

Catalog Number:

HK3005-01 (1 x 96 wells)

HK3005-02 (2 x 96 wells)

For Research Use Only. Not for use in diagnostic procedures.

v. 12.23

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## **INTENDED USE**

The Complement FHR-3 ELISA Assay Kit is to be used for the in vitro quantitative determination of FHR-3 in serum and plasma 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.

## **INTRODUCTION**

Human Complement Factor H-related protein 3 (FHR-3) is a crucial glycoprotein within the complement regulatory protein family. Synthesized mainly by the liver, FHR-3 is also produced by immune cells like monocytes and macrophages. This protein manifests in the plasma as multiple glycosylated forms, ranging between 35 to 56 kDa. Sharing structural similarities with Complement Factor H (CFH), FHR-3 is a significant player in the complement system's regulatory network, particularly within the alternative pathway (AP).

FHR-3's exact role in complement regulation remains to be fully deciphered. Preliminary studies suggest it has weak cofactor activity, potentially enhancing FH cofactor activity, and shows a limited inhibitory effect in hemolytic assays using FH-depleted serum. However, further research is needed to validate these findings.

In terms of clinical implications, aberrations in FHR-3 expression or function are associated with diseases such as atypical hemolytic uremic syndrome (aHUS), age-related macular degeneration (AMD), and systemic lupus erythematosus (SLE). In these conditions, improper complement activation and tissue deposition play a role in disease pathology. The role of FHR-3 in the AP, and its potential impact on disease mechanisms, highlights its significance as a potential therapeutic target in managing diseases influenced by complement dysregulation. Understanding FHR-3's involvement in these processes is vital for developing effective strategies to control complement-driven diseases.

FHR-3 can be measured with our HK3005 assay, designed to precisely quantify this intriguing glycoprotein.

## **PRINCIPLE OF THE ASSAY**

The human FHR-3 ELISA is a ready-to-use solid-phase enzyme-linked immunosorbent assay based on the sandwich principle with a working time of 1 hour and 15 minutes. 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 FHR-3. Peroxidase-conjugated antibody will bind to the captured FHR-3. Peroxidase-conjugate 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 FHR-3 standards (log). The human FHR-3 concentration of samples, which are run concurrently with the standards, can be determined from the standard curve.



**KIT FEATURES**

- Working time of 1 ¼ hours.
- Minimum concentration which can be measured is 0.16 ng/ml.
- Measurable concentration range of 0.16 to 10 ng/ml.
- Working volume of 100 µl/well.

**CROSS REACTIVITY**

Potential cross-reacting proteins detected in the Complement FHR-3 ELISA:

Cross reactant	Reactivity
FH (native)	Negative
FHR-1 (recombinant)	Negative
FHR-2 (recombinant)	Negative
FHR-4A (recombinant)	Negative
FHR-4B (recombinant)	Negative
FHR-5 (recombinant)	Negative

Table 1

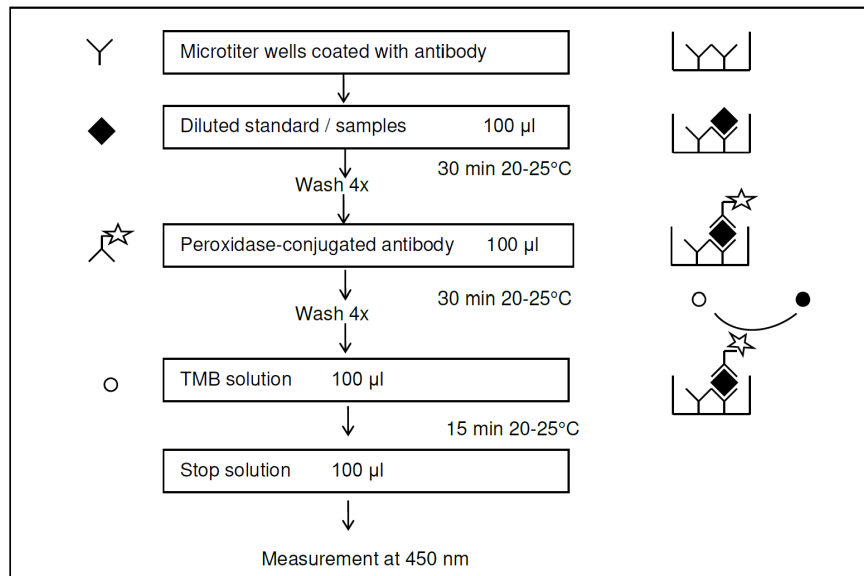
Potential cross-reacting species detected in the Complement FHR-3 ELISA:

Cross reactant	Reactivity
Rat	Negative
Mouse	Negative
Pig	Negative
Monkey (Cynomolgus)	Positive

Table 2

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

**PROTOCOL OVERVIEW**





- The Complement FHR-3 ELISA is a ready-to-use solid-phase enzyme-linked immunosorbent assay based on the sandwich principle with a working time of 1 hour and 15 minutes.
- 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 FHR-3.
- Peroxidase-conjugated antibody will bind to the captured FHR-3.
- Peroxidase-conjugate 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 FHR-3 standards (log).
- The human FHR-3 concentration of samples, which are run concurrently with the standards, can be determined from the standard curve.

#### KIT COMPONENTS AND STORAGE INSTRUCTIONS

Kit Component	Quantity HK3004-01	Quantity HK3004-02	Color Code
Wash buffer 20x	1 vial (60 ml)	1 vial (60 ml)	Colorless
Dilution Buffer 10x	1 vial (15 ml)	2 vials (15 ml)	Green
Standard	2 vials, lyophilized	4 vials, lyophilized	White
Peroxidase-conjugated antibody	1 vial, 1 ml lyophilized	2 vials, 1 ml lyophilized	Blue
TMB substrate	1 vial (11 ml)	1 vial (22 ml)	Brown
Stop solution	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 3

- Store the kit components at 2 - 8°C immediately upon receipt. Do not freeze.
- Do not use components beyond the expiration date printed on the kit label.
- The standard and detection antibody in lyophilized form are stable until the expiration date indicated on the kit label, if stored at 2 - 8°C.
- The exact quantity of the standard is specified on the vial label and the Certificate of Analysis.
- The standard is single use. After reconstitution it cannot be stored.



- 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 one month if stored at 2 - 8°C.

#### **MATERIALS REQUIRED 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.
- Centrifuge for 1 ml tubes.

#### **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 colorless 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 has been 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 guidelines for prevention of transmission of blood-borne infections.



- Note: Polymorphism of the protein FHR-3 can cause negative results in African Americans samples

## **SAMPLE PREPARATION**

The recommended sample medium is EDTA plasma. All samples should be kept on ice whilst performing the assay.

### **Collection and handling**

#### **Serum and Plasma**

Collect blood using normal aseptic techniques. Blood samples should be kept on ice. If serum is used, separate serum from blood after clotting at room temperature within one hour 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.

#### **Storage**

Store samples below -20°C, preferably at -70°C in polypropylene tubes. Storage at -20°C can affect recovery of human FHR-3. Preferable use samples within 1-2 hours after thawing. Avoid multiple freeze-thaw cycles which may cause loss of human FHR-3 activity and give erroneous results. Do not use hemolyzed, hyperlipemic, heat-treated or contaminated samples. Before performing the assay, samples should mixed gently. Prepare all samples (controls and test samples) prior to starting the assay procedure. Avoid foaming.

### **Dilution procedures**

#### **Serum or plasma samples**

Due to expected high levels of FHR-3 it is advised to apply a 40 – 150x dilution range for accurate measurement of serum or plasma samples with supplied dilution buffer in polypropylene tubes. This recommended dilution applies to healthy samples, the optimal dilution for diseased samples should be tested. Optimal dilution is dependent on sample quality and expected FHR-3 quantity and should be determined for the specific sample set/study.

#### **Comment regarding recommended sample dilution**

The mentioned dilution for samples is a minimum dilution and should be used as a guideline. The recovery of FHR-3 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 FHR-3.

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



### Guideline for dilution of samples

Please see Table 4 for recommended sample dilutions. Volumes are based on a total volume of at least 230  $\mu$ l of diluted sample, which is sufficient for one sample in duplicate in the ELISA. For dilution of samples we recommend to use at least 10  $\mu$ l of sample.

Dilution	Pre-Dilution	Amount of sample or pre-dilution required	Amount of dilution buffer required
10x	Not necessary	20 $\mu$ l (sample)	180 ul
40x	Not necessary	10 ul (sample)	390 ul
60x	Not necessary	10 ul (sample)	590 ul
80x	Recommended: 40x (see nr. 1)	100 ul (pre-dilution)	100 ul
100x	Recommended: 10x (see nr. 1)	20 ul (pre-dilution)	180 ul
125x	Recommended: 10x (see nr. 1)	24 ul (pre-dilution)	276 ul
150x	Recommended: 10x (see nr. 1)	20 $\mu$ l (pre-dilution)	280 ul

Table 4

### 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. In case 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 deionized water.

#### Dilution buffer

Prepare dilution buffer by mixing 15 ml of the 10x dilution buffer with 135 ml of distilled or de-ionized water, which is sufficient for 1 x 96 tests. In case 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 by pipetting the amount of dilution buffer mentioned on the CoA in the standard vial. Use the standard vial as Tube 1 in Figure 2. Prepare each FHR-3 standard in polypropylene tubes by serial dilution of the reconstituted standard with dilution buffer as shown in Figure 2\*. After reconstitution the standard cannot be stored for repeated use.

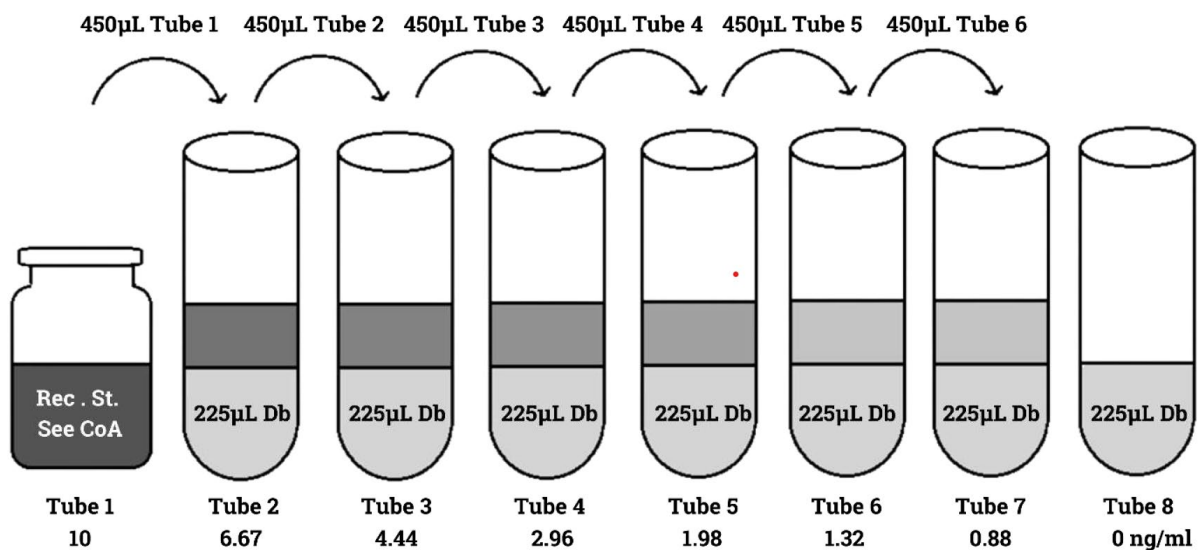


Figure 2

\*) CoA: Certificate of Analysis, St: Standard, Db: Dilution buffer

### Peroxidase-conjugated antibody

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

### 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 30 minutes 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 1x 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  $\mu$ l of diluted peroxidase-conjugated antibody 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 30 minutes at room temperature.
8. Repeat the wash procedure described in step 5a-e.
9. Add 100  $\mu$ l 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 15 minutes at room temperature. It is advised to control the reaction on the plate regularly. In the case of strong development the TMB reaction can be stopped sooner. Avoid exposing the microwell strips to direct sunlight. Covering the plate with aluminum foil is recommended.
11. Stop the reaction by adding 100ul of the stop solution with the same sequence and timing as used in step 9. 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.

#### **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.
- If the mean absorbance of samples exceeds that of the highest standard concentration, they fall outside the assay's range. Such samples ought to be reanalyzed using a greater dilution factor.

#### **TECHNICAL HINTS**

- User should be trained and familiar with ELISA assays and test procedure.
- If you are not familiar with the ELISA technique it is recommended to 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, detection antibody 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.

#### **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.



## Warranty Information

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at [info@eaglebio.com](mailto:info@eaglebio.com) or at 866-411-8023.