CFH (Human) IgG ELISA Kit

Catalog Number KA1477

48 assays

Version: 20

Intended for research use only

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Introduction

Intended Use

The CFH IgG ELISA Kit is intended for the quantitative detection of IgG antibodies against human complement factor H in human serum or plasma.

Background

Factor H is a complement regulatory glycoprotein that is found in human plasma in concentrations about 500 µg/mL. Its main function is the regulation of complement activation. Inhibitory autoantibodies against complement factor H resulting from an immunopathological reaction, dysregulate complement system.
Such autoimmune dysregulation of complement is associated with a specific form of atypical hemolytic uremic syndrome (AI-HUS). It is recommended testing anti-complement factor H autoantibodies in all cases of HUS at the onset of the disease. Approximately 30% of AI-HUS patients had diarrhoea as prodromal syndromes, which in turn are the typical sign in the classic form of HUS which is caused by Shigga toxin positive species of *E. coli*. Removal of anti-factor H antibodies from the bloodstream by plasmapheresis or the use of immune suppressive drugs to eliminate the antibody production is beneficial for the outcome of the disease.

Principle of the Assay

The CFH IgG ELISA Kit is an enzyme linked immunosorbent assay designed to detect IgG antibodies against complement factor H. The wells of the microtitrate plate are coated with purified human complement factor H. Antibodies against factor H present in serum sample bind to the immobilized factor H. Other antibodies, unbound to the factor H, are washed away during the next step. Then anti-human IgG antibodies labelled with horseradish peroxidase are added and those detect the antibodies from the sample that previously bound to factor H. The unbound labelled antibodies are washed away and the remaining labelled antibodies are visualized with a chromogenic substrate. The peroxidase activity leads to a change in colour of the solution. The reaction is stopped by adding an acidic solution. The colour intensity is directly proportional to the amount of anti-factor H antibodies in the sample.

General Information

Materials Supplied

List of component

Component	Amount
ELISA strips coated with human factor H purified from human plasma.	48 (8 x 6 strips) wells
Anti-CFH IgG standard (10,000 AU/mL, Artificial units/mL).	50 µL
IgG-HRP conjugate, 101x concentrated.	0.1 mL
Wash buffer 10x concentrated.	55 mL
Dilution buffer (DB) r.t.u.*	60 mL
Chromogenic substrate (TMB substrate) r.t.u. *	13 mL
Stop solution r.t.u. *	13 mL

* r.t.u. - ready to use

Note: Control sera may be colorless to yellowish or blue due to the use of different diluents.

Storage Instruction

- ✓ The ELISA kit should be used within three months after opening.
- ✓ Store the kit and the kit reagents at 2 to 10°C, in a dry place and protected from the light Under these conditions, the expiration period of the entire kit is indicated on the central label on the kit package, the expiration date of the individual components is indicated on their package.
- ✓ Put unused strips back in the package and seal or close tightly in a zippered bag with desiccant.
- ✓ The kit are transported refrigerated in thermal bags, transport time tup to 72 hours has no influence on expiration. If, upon receipt of the kit, you notice serious damage to the packaging of any component of the kit, inform the manufacturer immediately.
- ✓ Store unused test samples undiluted, aliquoted and frozen at -18°C to -28°C. Frequent freezing and thawing is not recommended. If you store samples at 2°C to 10°C, then test them within one week.
- ✓ Test sample solutions at the working concentration cannot be stored. Always prepare them fresh.

Materials Required but Not Supplied

- ✓ Distilled/deionized water
- ✓ Precision micropipettes 20, 200 and 1000 µL and suitable tips
- ✓ Graduated cylinders (1000 mL)
- ✓ Microplate washer or other device for microplate washing
- ✓ Absorbent papers
- ✓ ELISA reader
- ✓ Adhesive membrane or microplate lid to cover the wells during incubations

Note: It is recommended to use a precise dispenser e.g. Multipette Xtream Eppendorf for the dispensing of the TMB-BF and Stop solution.

Precautions for Use

✓ Safety Precautions

- All ingredients of the kit are intended for laboratory use only.
- Wash buffer, chromogenic substrate, stop solution, and dilution buffer are interchangeable between Elisa kits, unless otherwise noted in the kit instructions.
- Work aseptically to avoid microbial contamination of samples and reagents.
- When collecting, diluting, and storing reagents, be careful not to cross-contaminate them or contaminate them with enzymatic activity inhibitors.
- The chromogenic substrate shouldn't come into contact with oxidizing agents and metal surfaces. Because it is sensitive to light, close the bottle immediately after use. The chromogenic substrate must be clear in use. Do not use the solution if it is blue.
- Follow the instruction manual exactly. Non-reproducible results may arise in particular:
  * Insufficient mixing of reagents and samples before use.
  * Inaccurate pipetting and inadequate incubation times
  * Poor washing technique or spilling the rim of well with sample or with HRP-conjugate
  * Using the same tip when pipetting the different solutions or swapping caps.
- Human control sera and standard used in the kit were tested for the absence of HBsAg, anti-HIV-1,2 and anti-HCV antibodies. Treat test specimens, control sera, standards, and use strips as infectious material. Autoclave items that have been in contact with them for 1 hour at 121°C or disinfect for at least 30 minutes with 3% chloramine solution.
- Neutralize liquid waste containing stop solution (sulfuric acid solution) with 4% sodium bicarbonate solution before disposal.
- Disinfect the waste generated during strip washing in a waste container using a suitable disinfectant solution (e.g. Incidur, Incidin, chloramine, …) at the concentration recommended by the manufacturer.
- Handle stop solution carefully to avoid splashing on the skin or mucous membranes. If this happens, wash the affected area with plenty of running water.
- The test procedure requires qualified laboratory personnel.
- Do not smoke, eat or drink during work. Do not pipette by mouth, use suitable pipetting device. Wear protective gloves and wash your hands thoroughly after work. Be careful not to spill specimens or form an aerosol.
- All reagents and packaging material must be disposed of in accordance with applicable legislation.
- In case of suspicion of an adverse event in connection with the use of the kit inform the manufacturer and the competent state authority without delay.
- Standard, conjugate 101x concentrated and dilution buffer are preserved with ProClin 300® (mix of 5-

Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H-iothiazol-3-one (3:1)). Therefore, the following warnings and safety precautions apply to these solutions:

H317 May cause an allergic skin reaction.

H411 Toxic to aquatic life with long lasting effects.

P280 Wear protective gloves/protective clothing/ protective glasses/ face protection.

P302+P352 OF ON SKIN: Wash with plenty of water.

P333+P313 If skin irritation or rash occurs: Get medical advice/attention.

P362+P364 Take off contaminated clothing and wash it before reuse.

Assay Protocol

Reagent Preparation

Allow all the kit components to reach room temperature (~ 20 min). Mix all reagents well before use to ensure homogeneity.

✓ Wash Buffer

Prepare Wash Buffer by diluting the Wash Buffer concentrate 10 times with an appropriate volume of distilled or deionized water (e.g. 50 mL of the concentrated Wash buffer + 450 mL of distilled water).

Note: If there are crystals of salt present in the concentrated Wash buffer, warm up the vial to 32 to 37°C in a water bath. Diluted Wash buffer is stable for one month if stored at room temperature.

✓ Detection Antibody anti-IgG Conjugated to HRP (IgG-HRP)

Dilute the IgG-HRP concentrate 101x with the Dilution buffer. For one 8-well strip prepare 1 mL of the IgG-HRP conjugate solution.

If you intend to prepare a certain amounts of the IgG-HRP solution see the recommendations indicated in table below. *Note: Do not store the diluted IgG-HRP.*

Number of 8-well strips	IgG-HRP conjugate concentrate 101x (µL)	Dilution buffer (mL)
2	20	2
3	30	3
4	40	4
5	50	5
6	60	6

✓ Standard anti-CFH IgG

Prepare the serial dilutions of the Standard as follows:

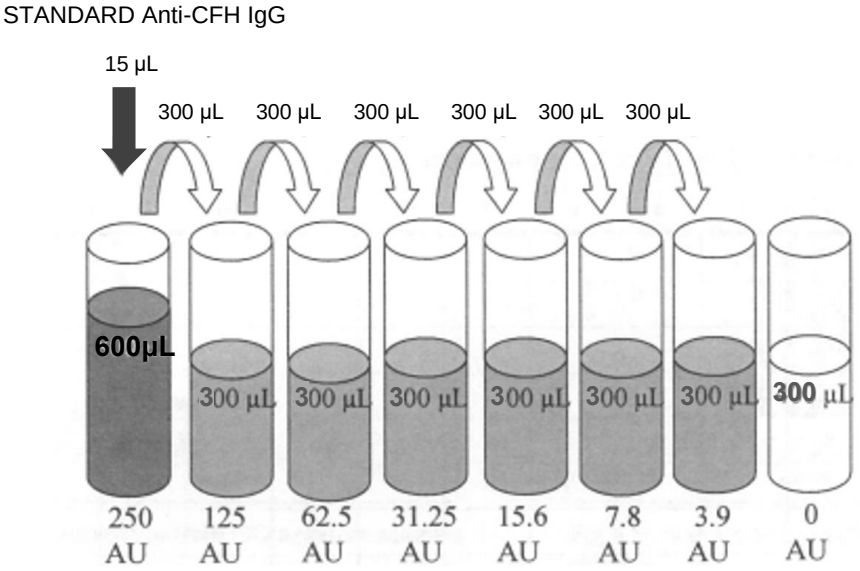
Prepare eight 1.5 mL PP microtubes and label them as 250 AU, 125 AU, 62.5 AU, 31.25 AU, 15.6 AU, 7.8 AU, 3.9 AU, 0 AU. Pipette sequentially 600 µL, 300 µL, 300 µL, 300 µL, 300 µL, 300 µL, 300 µL, 300 µL of the Dilution buffer to the microtubes. Mix well the Standard stock solution and pipette 15 µL of the stock solution to the first microtube (250 AU/mL). Mix the content of the tube, remove 300 µL and add it

to the second tube (125 AU/mL). Then continue with dilutions as 300 + 300 µL to prepare the entire set of concentrations ranging from 250 AU/mL to 3.9 AU/mL. Do not add any anti-CFH into the tube labelled with 0, the zero standard is the Dilution buffer only.

Note: Change the pipette tip after each dilution, always mix well the content.

For easier understanding of the standard dilution procedure look at the scheme bellow (Dilution of the anti-CFH standard).

Figure No. 1 Dilution of the anti-CFH standard



Sample Preparation

- ✓ Store serum, plasma samples frozen at -18°C or lower.
- ✓ Thaw plasma samples quickly in a water bath at 37°C, the plasmatic proteins may precipitate if thawed slowly. Thaw serum samples either in a water bath at 37°C or at the laboratory temperature.
- ✓ Dilute the samples 101x with the Dilution buffer (e.g. 5 µL sample + 500 µL Dilution buffer).
- ✓ Prepare enough volume to measure each diluted sample in replicates 100 µL/well.
- ✓ If you expect anti-CFH concentrations higher than 250 AU/mL dilute the samples with the Dilution buffer to obtain the concentrations that will fall within the standard range (250-3.9 AU/mL).
- ✓ Do not dilute Dilution buffer, TMB solution, Stop solution! They are ready to use.

Assay Procedure

Manufacturer will not be held responsible for results if manual is not followed exactly.

1. Allow all the kit components to reach temperature (~ 20 min).
2. Prepare the working concentrations (in the volume needed) of Wash buffer and of the IgG-HRP solution.
3. Dilute the anti-CFH IgG standard to concentrations 250, 125, 62.5, 31.25, 15.6, 7.8, 3.9 and 0 AU/mL.
4. Dilute the samples 101x with the Dilution buffer.
5. Open the aluminium bag containing the strips and remove the desired number of strips. Put the unused strips together with the desiccant to the provided plastic bag and seal it. Store the unused strips at + 2 to + 10°C.
6. Pipette 100 µL of standards (0-250 AU/mL) and samples to the wells (see Plate Layout).
7. Cover the strips with the sealing membrane or with a lid. *Note: The cover prevents evaporation from the wells during the incubation.* Incubate for 1 hour (+/- 5 minutes) at laboratory temperature.
8. Aspirate and wash 5 times with 250-400 µL of the Wash buffer. Invert and tap the plate on a pile of absorbent papers (see Safety precautions).
9. Pipette 100 µL of the diluted IgG-HRP into each well. Incubate for 1 hour (+/- 5 minutes) at laboratory temperature.
10. Aspirate and wash 5 times with 250-400 µL of the Wash buffer. Invert and tap the plate on a pile of absorbent papers (see Safety precautions).
11. Pipette 100 µL of TMB-BF solution to the wells. Incubate in dark place for 20 minutes (+/- 1 minute) at laboratory temperature.
12. Pipette 100 µL of Stop solution to the wells.
13. Tap the microplate side gently to ensure complete mixing of the TMB with the Stop solution.
14. Read the absorbance at 450 nm, it is recommended to use a reference reading 620-690 nm.

✓ Flow Chart

Prepare the working concentrations of reagents, standards and dilute samples

↓

Pipette 100 µL of standards and the diluted samples to the wells

↓

Incubate 60 minutes at room temperature

↓

Wash 5 times (250-400 µL/well), aspirate

↓

Pipette 100 µL of the diluted IgG-HRP

↓

Incubate 60 minutes at room temperature

↓

Wash 5 times (250-400 µL/well), aspirate

↓

Dispense 100 µL/well of TMB substrate

↓

Incubate 20 minutes in dark at room temperature

↓

Dispense 100 µL/well of Stop solution

↓

Read the absorbance at 450/ 620-690 nm within 10 minutes

Data Analysis

Calculation of Results

✓ Processing of Results

- Subtract the absorbance at the reference wavelength from the absorbance at 450 nm (usually performed automatically by the ELISA reader).
- Compute means in duplicates.
- Subtract the Standard 0 mean from all of the other mean values (Blank Difference data). If the absorbance of controls or tested sera are negative after background subtraction, consider them as zero value.
- Construct the standard curve by plotting the mean absorbance for each standard (Blank Difference data) on the y-axis versus the concentration in AU/mL on the x-axis. Draw the best fit curve through the standard points.

  Note: Regression is linear only if the range is narrowed to 125 AU/mL as a maximum, if you wish to use the entire set of concentration as the standard points (3.9-250 AU/mL) you will need to use a different fitting algorithm suitable for ELISA type data (i.e. cubic spline, 4PL algorithm).
- Compute the concentrations of anti-CFH IgG in AU/mL in samples according to the standard curve formula.

✓ Interpretation of Results

Samples with concentrations lower than 3.9 AU/mL (the lowest standard) interpret as <3.9 AU/mL of anti-CFH IgG. Samples with concentrations higher than 250 AU/mL interpret as >250 AU/mL or dilute them with Dilution buffer and repeat the test with the diluted samples, e.g. 202x a 404x (multiply the final measured concentration with the dilution factor, i.e. 2x or 4x).

To characterize the sample as anti-CFH IgG positive or negative, it is suitable to determine your own cut-off value. Cut-off value depends on the chosen population group.

Use your own routinely used calculation or our recommended procedure:

The cut-off value is determined from expected anti-CFH IgG negative samples (e.g. blood donors).

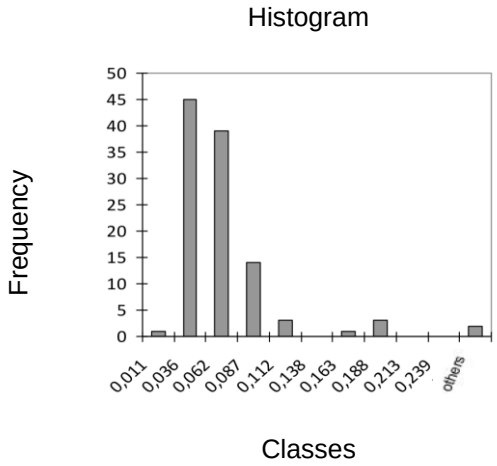
Calculation of the cut-off value:

a. Make histogram graph from your samples. Check if the data has normal distribution (Gaussian). If not, compute logarithm of OD (subtract blank) before the data processing. Then check again the normal distribution.
b. Compute the OD mean of all negative samples
c. Compute standard deviation from negative samples
d. Compute cut-off using formula:
   OD mean + 3 x standard deviation
e. Compute cut-off in AU/mL using your actual calibration curve.

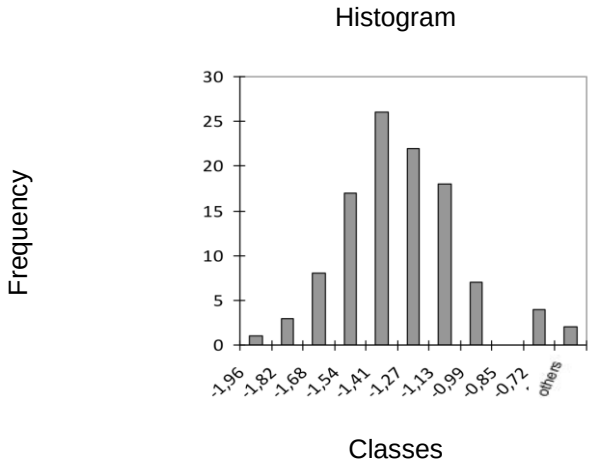
Example of cut-off value for the population from Czech Republic:

Cut-off for serum samples was calculated from 107 samples (healthy persons - 59 (55%) men and 49 (45%)

women, average age 31 years). The OD values has non-Gaussian distribution, therefore the logarithm of OD was used.



Histogram of from OD negative samples

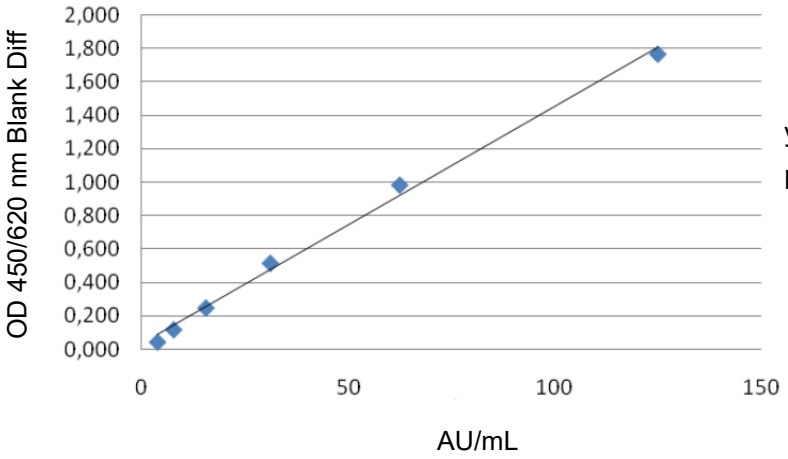


Histogram of logarithm of OD from negative samples

The calculated cut-off was 27 AU/mL for serum samples, 18 AU/mL for plasma samples. Do not use these values for the interpretation of your results.

- Standard curve

Figure No. 2 Standard curve example (3.9 – 125 AU/mL). Do not use this curve for calculation of AU/mL from your data.



Performance Characteristics

✓ Validity criteria

The mean absorbance values of Standards S 0, S 250 and the difference between the Standards S 3,9 and S 0 are in the ranges stated in the Quality control certificate for this kit lot.

✓ Intraassay

(N = number of tests within the plate):

N	sample	AVG AU/mL	± σ	CV (%)
27	serum B	5	0.48	9
24	serum A	266	14.3	5

✓ Interassay

(N= number of test repetitions)

N	sample dilutions	AVG AU/mL	± σ	CV (%)
8	serum A 404x	213	22	10
8	serum A 808x	122	17	14
8	serum A 1616x	66	10	15
8	serum A 3232x	36	4	12

✓ Linearity

Two positive samples were assayed in dilution 101x and also in serial dilutions that ranged from 202x to 6464x.

	Sample dilution	Observed (AU/mL)	Expected (AU/mL)	O/E (%)
Sample 1	101x	>MAX	-	-
	202x	>MAX	-	-
	404x	146	-	-
	808x	80	73	110
	1616x	41	37	111
	3232x	21	18	113
	6464x	12	9	128
Sample 2	101x	>MAX	-	-
	202x	264	-	-
	404x	137	132	104
	808x	71	66	108
	1616x	34	33	103
	3232x	18	17	111
	6464x	8	8	102

✓ Interference

Haemolytic and lipemic samples have no influence on the test results up to the concentration of 50 mg/mL of haemoglobin, 5 mg/mL of bilirubin and 50 mg/mL of triglycerides.

✓ Sensitivity and specificity of the test

Specificity was determined using 130 serum samples (blood donors), where we did not expected anti-CFH IgG antibodies. Specificity of the test was 98.5%.

Sensitivity was determined using 9 samples from persons with DEAP-HUS (deficiency of CFHR plasma proteins and factor H autoantibody positive HUS) confirmed by genetic tests. Sensitivity was 100%.

Resources

References

1. Anti-Factor H autoantibodies associated with atypical hemolytic uremic syndrome. Dragon-Durey MA, Loirat C, Cloarec S, Macher MA, Blouin J, Nivet H, Weiss L, Fridman WH, Frémeaux-Bacchi V. J Am Soc Nephrol. 2005 Feb;16(2):555-63.
2. Successful pre-transplant management of a patient with anti-factor H autoantibodies-associated haemolytic uraemic syndrome. Kwon T, Dragon-Durey MA, Macher MA, Baudouin V, Maisin A, Peuchmaur M, Fremeaux-Bacchi V, Loirat C. Nephrol Dial Transplant. 2008 Jun;23(6):2088-90.
3. Factor H autoantibodies in atypical hemolytic uremic syndrome correlate with CFHR1/CFHR3 deficiency. Józsi M, Licht C, Strobel S, Zipfel SL, Richter H, Heinen S, Zipfel PF, Skerka C. Blood. 2008 Feb 1;111(3):1512-4.
4. Anti-factor H autoantibody-associated hemolytic uremic syndrome: review of literature of the autoimmune form of HUS. Dragon-Durey MA, Blanc C, Garnier A, Hofer J, Sethi SK, Zimmerhackl LB. Semin Thromb Hemost. 2010 Sep;36(6):633-40.

Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	Standard 0	Standard 0	Sample 1	Sample 1								
B	Standard 3.9	Standard 3.9	Sample 2	Sample 2								
C	Standard 7.8	Standard 7.8	Sample 3	Sample 3								
D	Standard 15.6	Standard 15.6	Sample 4	Sample 4								
E	Standard 31.3	Standard 31.25	…	…								
F	Standard 62.5	Standard 62.5										
G	Standard 125	Standard 125										
H	Standard 250	Standard 250										