ELISA-VIDITEST
anti-complement factor H

REF ODZ-166 Σ 48 tests 2°C 10°C 2°- 10 °C

Type of determination: IgG antibodies

Type of evaluation: Quantitative

Type of samples: Serum/Plasma

Processing: Manual

IVD

VIDIA

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Instructions for use

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1. TITLE

ELISA-VIDITEST anti-complement factor H

2. INTENDED USE

ELISA-VIDITEST anti-complement factor H is intended for professional use for the quantitative detection of IgG antibodies against human complement factor H in human serum or plasma. 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 haemolytic 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.

3. TEST PRINCIPLE

ELISA-VIDITEST anti-complement factor H 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.

4. KIT COMPONENTS

ELISA strips coated with human factor H purified from human plasma STRIPS Ag	1 x 6 strips
50 µL Anti-CFH IgG standard (10.000 AU/mL[1)]) STANDARD	1 vial
0.1 mL IgG-HRP conjugate - detection anti-human antibody labelled by horseradish peroxidase,101x concentrated CONJ 101x	1 vial
55 mL Wash buffer 10x concentrated WASH 10x	1 vial
60 mL Dilution buffer (DB) r.t.u. [2)] DIL	1 vial
13 mL Chromogenic substrate (TMB-BF substrate) r.t.u. (TMB/H_2O_2) TMB-BF	1 vial
13 mL Stop solution r.t.u. (0.4 M sulfuric acid) STOP	1 vial
Instruction manual	
Quality control certificate	

[1)] AU/ml (Arteficial units/ml)

[2)] r.t.u. ready to use

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

Chromogenic substrate TMB-BF is compatible and interchangeable between ELISA-VIDITEST kits which contain TMB-BF and not with other Chromogenic substrates TMB, TMB-O.

5. MATERIAL NEEDED BUT NOT PROVIDED

Distilled/deionised water; precision micropipets 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.

6. PREPARATION OF REAGENTS

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 deionised water (e.g. 50 mL of the concentrated Wash buffer + 450 mL of distilled water).

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 1. Do not store the diluted IgG-HRP.

Table 1.

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

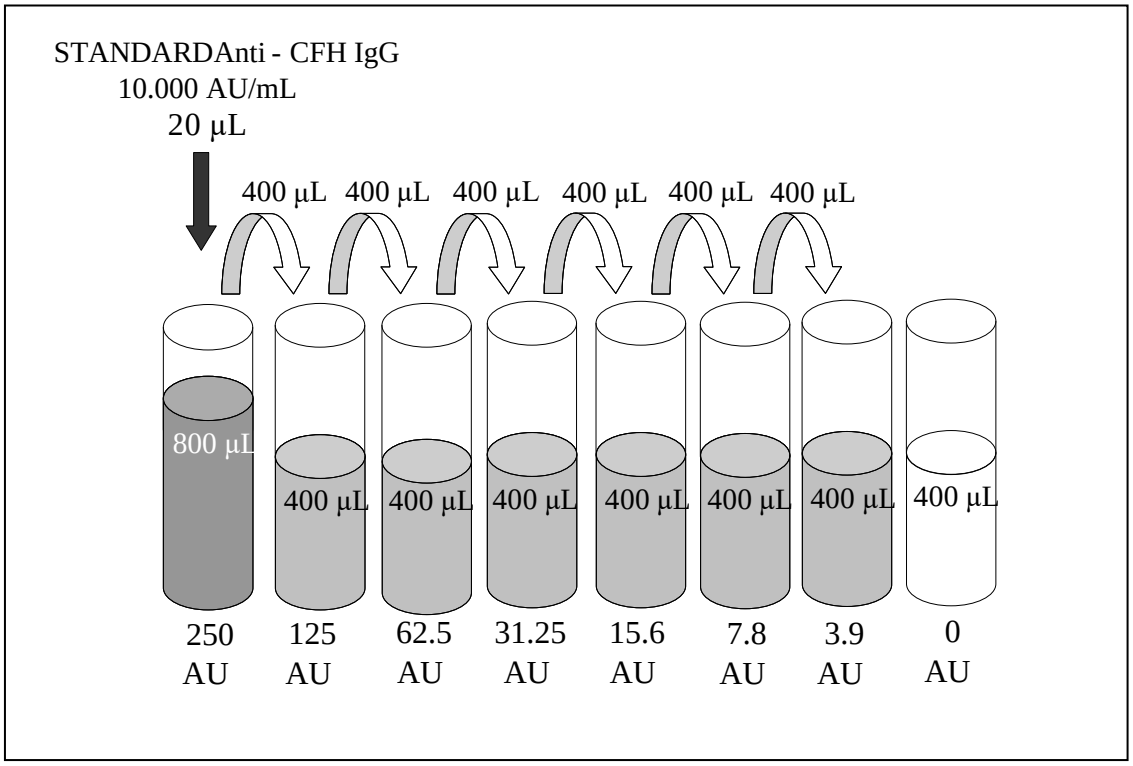
STANDARD Anti-CFH IgG

Standard is supplied as 10.000 AU/mL stock solution. 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 800 µL, 400 µL, 400 µL, 400 µL, 400 µL, 400 µL, 400 µL, 400 µL of the Dilution buffer to the microtubes. Mix well the Standard stock solution and pipette 20 µl of the stock solution to the first microtube (250 AU/mL). Mix the content of the tube, remove 400 µl and add it to the second tube (125 AU/mL). Then continue with dilutions as 400 + 400 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. *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



SAMPLES

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 (eg. 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-BF solution, STOP solution! They are ready to use.

7. ASSAY PROCEDURE

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

a. Allow all the kit components to reach temperature (~ 20 min).

b. Prepare the working concentrations (in the volume needed) of Wash buffer and of the IgG-HRP solution.

c. Dilute the anti-CFH IgG standard STANDARD to concentrations 250, 125, 62.5, 31.25, 15.6, 7.8, 3.9 and 0 AU/mL.

d. Dilute the samples 101x with the Dilution buffer DIL.

e. 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.

f. Pipette 100 µL of standards (0 – 250 AU/mL) and samples to the wells (see Pipetting scheme).

g. Cover the strips with the sealing membrane or with a lid. T*he cover prevents evaporation from the wells during the incubation.* Incubate for 1 hour (+/-5 minutes) at laboratory temperature.

h. 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).

i. Pipette 100 µL of the diluted IgG-HRP CONJ into each well. Incubate for 1 hour (+/-5 minutes) at laboratory temperature.

j. 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).

k. Pipette 100 µL of TMB-BF solution TMB-BF to the wells. Incubate in dark place for 20 minutes (+/- 1 minute) at laboratory temperature.

l. Pipette 100 µL of STOP solution STOP to the wells.

m. Tap the microplate side gently to ensure complete mixing of the TMB-BF with the STOP solution.

n. Read the absorbance at 450 nm, it is recommended to use a reference reading 620-690 nm.

Figure No. 2 Pipetting scheme (S = STANDARD, P = patient sample):

	1	2	3	4	5	6	7	8	9	10	11	12
A	S 0	S 0	P1	P1								
B	S 3.9	S 3.9	P2	P2								
C	S 7.8	S 7.8	P3	P3								
D	S 15.6	S 15.6	P4	P4								
E	S 31.3	S 31.3	...	...								
F	S 62.5	S 62.5										
G	S 125	S 125										
H	S 250	S 250										

8. 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.

9. 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*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.

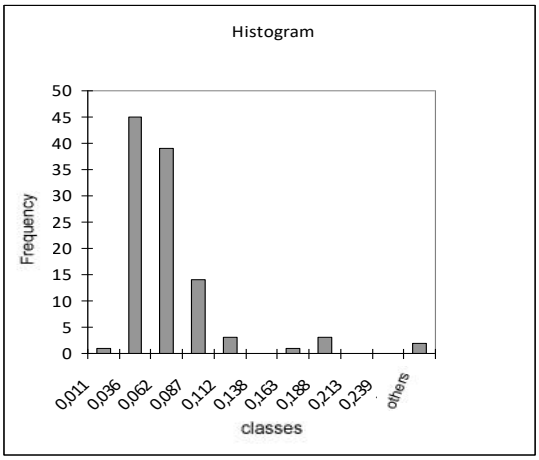


Fig.3 Histogram of from OD negative samples

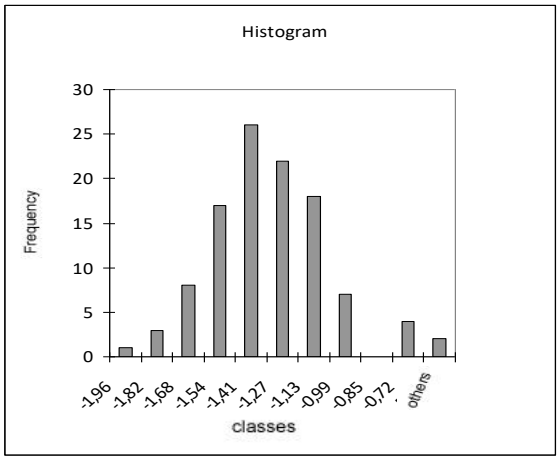
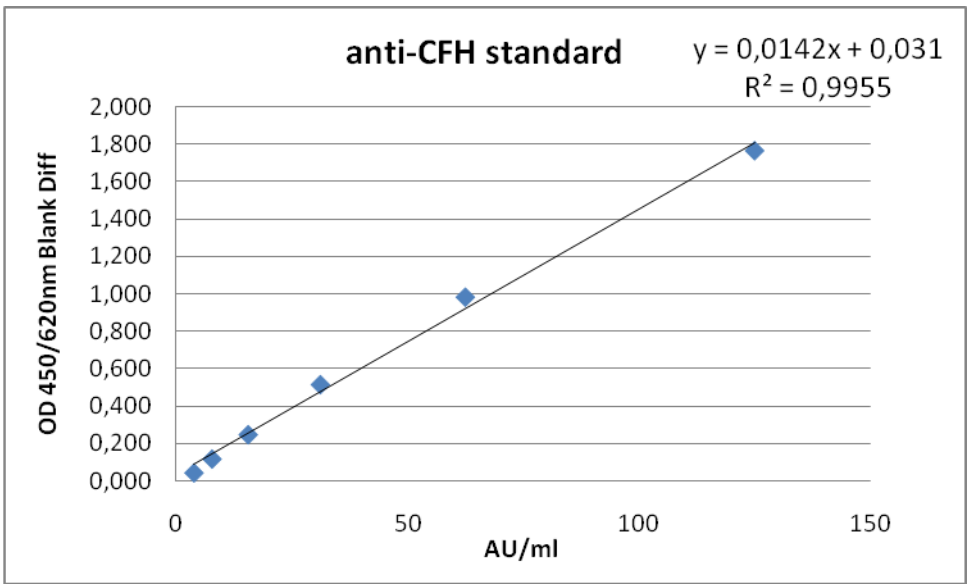


Fig.4 Histogram of logarithm of OD from negative samples

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.

10. TEST CHARACTERISTICS

Figure No. 5: Standard curve example (3.9 – 125 AU/ml). Do not use this curve for the calculation of AU/mL from your data.



10.1 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.

10.2 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

10.3 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

10.4 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

10.5 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.

10.6 Diagnostic sensitivity and specificity of the test

Diagnostic 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 %.

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

10.7 Analytical sensitivity of the test

The analytical sensitivity of the assay is defined as the mean of the sample without analyte plus three times of the standard deviation and represents the lowest detectable antibody titer. The analytical sensitivity value is determined for each kit lot and is stated in the **Quality Control Certificate** of that kit lot.

10.8 Analytical specificity of the test

The quality of the purified human complement factor H, which recognizes specific antibodies in patient samples, ensures the high specificity and sensitivity of this assay. However, to determine the diagnosis, the test results must always be interpreted in the context of clinical signs and the results of other laboratory tests, see RESULT INTERPRETATION.

10.9 Measuring range

The measuring range is determined by the measuring capability of the spectrophotometer / colorimeter used.

10.10 Limit of quantification

The limit of quantification is defined as the lowest measurable concentration that can be distinguished from zero with 95% confidence. This value is determined for each batch of the kit and is stated in the **Quality Control Certificate** of the given batch of the kit.

11. WARNINGS

a. All kit components are for laboratory use only.

b. The manufacturer guarantees the usability of the kit as a whole.

c. Wash buffer WASH 10x, Urea solution UREA, Chromogenic substrate TMB-BF, Stop solution STOP, and Dilution buffer DIL are interchangeable between ELISA-VIDITEST kits, unless otherwise noted in the kit instructions.

d. Work aseptically to avoid microbial contamination of samples and reagents.

e. When collecting, diluting, and storing reagents, be careful not to cross-contaminate them or contaminate them with enzymatic activity inhibitors.

f. The Chromogenic Substrate TMB-BF 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 TMB-BF must be clear in use. Do not use the solution if it is blue.

g. Follow the Instruction manual exactly. Non-reproducible results may arise in particular:
   - insufficient mixing of reagents and samples before use
   - inaccurate pipetting and non-compliance with the incubation times given in Chapter 7
   - poor washing technique and splashing of the edges of the wells with sample or conjugate
   - using the same tip when pipetting different solutions or swapping caps

h. Human control sera and standards used in the kit were tested for the absence of HBsAg, HCV and anti-HIV-1,2 antibodies. Treat test specimens, control sera, standards, and used 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.

i. Neutralize liquid waste containing Stop solution (sulfuric acid solution) with 4% sodium bicarbonate solution before disposal.

j. Disinfect the waste generated during strip washing in a waste container using a suitable disinfectant solution (eg Incidur, Incidin, chloramine, ...) at the concentration recommended by the manufacturer.

k. Handle Stop solution STOP carefully to avoid splashing on the skin or mucous membranes. If this happens, wash the affected area with plenty of running water.

l. Do not eat, drink or smoke while working. Do not pipette by mouth, but by suitable pipetting devices. Wear protective gloves and wash your hands thoroughly after work. Be careful not to spill specimens or form an aerosol.

m. All reagents and packaging material must be disposed of in accordance with applicable legislation.

n. 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.

12. SAFETY PRECAUTIONS

Standard STANDARD, conjugate 101x concentrated CONJ 101x and Dilution buffer DIL are preserved with ProClin 300 (a mixture of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H-isothiazol-3-one (3:1)). Therefore, the following warnings and safety precautions apply to these solutions:

Warning	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.

Further information can be found in the safety data sheet.

13. STORAGE AND EXPIRATION

It is recommended to use the kit within three months after opening.

a. Store the kit and the kit reagents at +2 °C 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.

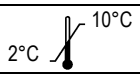
b. Put unused strips back in the package and seal or close tightly in a zippered bag with desiccant.

c. The kits are transported refrigerated in thermal bags, transport time up 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.

d. 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.

e. Test sample solutions at the working concentration cannot be stored. Always prepare them fresh.

14. USED SYMBOLS

Symbol	Explanation
	number of tests
CE	Conformité Européenne – product meets the requirements of European legislation
IVD	diagnostics *in vitro*
±σ	standard deviation
CV	coefficient of variation
OD	optical density
	manufacturer
	expiration
LOT	lot of kit
	storage at +2 °C - +10 °C
°C	Celsius degree
%	percentage
n	number of tested samples
A	value of a certain sample
	read the package leaflet
REF	catalog number

15. FLOW CHART

Step 1	**Prepare the working concentrations of reagents, standards and dilute samples**
	↓
Step 2	**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
	↓
Step 3	**Pipette 100 µL of the diluted IgG-HRP**
	↓
	Incubate 60 minutes at room temperature
	↓
	Wash 5 times (250-400 µL/well), aspirate
	↓
Step 4	**Dispense 100 µL/well of TMB-BF substrate**
	↓
	Incubate 20 minutes in dark at room temperature
	↓
Step 5	**Dispense 100 µL/well of Stop solution**
	↓
Step 6	**Read the absorbance at 450/ 620-690 nm within 10 minutes**

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