AB Diagnostic Systems

REF **DK.076.01.8** Σ **96**

REF **DK.076.05.8** Σ **480**

IVD **For *In vitro* Diagnostic Use**

INSTRUCTIONS FOR USE
abia HCV Ab express
Enzyme immunoassay for detection of antibodies to Hepatitis C virus

This Package Insert provides information for Professional Use of the kit.
Format 1 contains sufficient reagents for 96 assays (one breakable plate), including controls; the kit is intended for manual testing with a possibility of fractional (one strip) use of the kit or for the simultaneous 96 assays on the open automated analyzers; triple testing (32 x 3) with the open automated analyzers is possible.
Format 2 contains sufficient reagents for 480 assays (five breakable plates), including controls; the kit is intended for manual testing with a possibility of fractional use of the kit or for the simultaneous 480 assays (96x5) on the open automated analyzers.

I. INTENDED USE

The abia HCV Ab express kit is an enzyme immunoassay for detection of IgG and IgM antibodies to Hepatitis C virus (HCV) in human blood serum (plasma), immunoglobulins and other diagnostic human serum (plasma) based products. It is recommended for primary laboratory diagnosis of Hepatitis C virus and donor screening.

This kit is for diagnostic use by a trained laboratory professional and will not be sold to the general public. All the reagents are for professional *in vitro* diagnostic use only.

The results of this or any other diagnostic assay should be used and interpreted only in the context of the overall clinical picture.

II. CONTENTS OF THE abia HCV Ab express

Table 1

LABEL	NATURE OF THE REAGENTS	PRESENTATION	
		Format 1	Format 2
HCV Ag Coated Plate	Polystyrene stripped 96-well plate (breakable wells) coated with mixture of recombinant antigens – core, NS3, NS4, NS5 HCV. Store at 2-8 °C until expiration date.	1 plate	5 plates
Conjugate	Mixture of antibodies to human IgG and IgM, conjugated with horseradish peroxidase. Transparent or opalescent liquid, from yellow to orange. Preserving agent: 0.10% ProClin 300, 0.001% gentamicin sulfate. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 14.0 ml	3 vials 25.0 ml or 1 vial 60.0 ml
Positive Control, Inactivated	Heat inactivated human serum (plasma) positive for antibodies to Hepatitis C virus, negative for HBsAg and anti-HIV-1,2. Transparent or slightly opalescent liquid, crimson colored. Preserving agents: 0.20% sodium azide, 0.04% ProClin 300. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 3.0 ml	2 vials 3.0 ml or 1 vial 5.0 ml
Negative Control, Inactivated	Heat inactivated human serum (plasma) negative for antibodies to Hepatitis C virus, HBsAg and anti-HIV-1,2. Transparent or slightly opalescent liquid, green colored. Preserving agents: 0.19% sodium azide, 0.04% ProClin 300, 0.001% gentamicin sulfate. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 3.0 ml or 1 vial 5.0 ml	4 vials 3.0 ml or 2 vials 5.0 ml
Sample Diluent	Pink colored liquid. Sedimentation is possible. Preserving agents: 0.05% sodium azide, 0.13% ProClin 300. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 20.0 ml	2 vials 20.0 ml

Washing Solution (concentrated 25-fold)	Transparent or slightly opalescent liquid, colorless or pale yellow, sediment may form that dissolves at 35-39 °C and shaking. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 50.0 ml or 1 vial 120.0 ml	4 vials 50.0 ml or 2 vials 120.0 ml
TMB-Substrate	Tetramethylbenzidine in citric acid buffer, containing H_2O_2. Transparent colorless liquid, coloration is possible. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 14.0 ml	3 vials 25.0 ml or 1 vial 60.0 ml
	or	**or**	**or**
Substrate Buffer	Citric acid solution, pH 4.1-4.3, containing H_2O_2. Transparent colorless liquid. Preserving agent: 0.04% ProClin 300. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 25.0 ml	3 vials 25.0 ml or 2 vials 50.0 ml
	and		
TMB (concentrated 11-fold)	Solution containing 3,3',5,5'-Tetramethylbenzidine (0.1%) and dimethyl sulfoxide (DMSO) (84.50%). Transparent colorless liquid. Coloration is possible. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 2.5 ml	3 vials 2.5 ml or 2 vials 3.5 ml
Stopping Reagent	Sulfuric acid solution (H_2SO_4) 0.2M. Transparent colorless liquid. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 25.0 ml or 1 vial 50.0 ml	4 vials 25.0 ml or 2 vials 50.0 ml
Plate lid or Protective films for EIA plates		1 2	5 10
Pack clip or polyethylene bag with a Zip-Lock		1	3
Disposable plastic dishes for liquid reagents		2	10
Disposable tips		16	80

III. PRECAUTIONS

The reliability of the results depends on correct implementation of the following requirements:

- The temperature in the lab should be 18-24 °C.
- Inspect the contents of the box: check the vials and labels integrity. In case of label loss or labels/vials damage, vials should be disposed of and **the kit cannot be used.**
- Do not use expired reagents.
- Do not mix reagents from different lots within a given test run.
- Carefully reconstitute the reagents avoiding any contamination.
- Do not carry out the test in the presence of reactive vapors (acid, alkaline, aldehyde vapors) or dust that could alter the enzyme activity of the conjugates.
- Use glassware thoroughly washed and rinsed with deionized water or preferably, disposable material.
- Do not allow the microplate to dry between the end of the washing operation and the reagent distribution.

- Do not store the plate for more than 10 minutes between individual operations. Do not let the plate wells dry.
- The enzyme reaction is very sensitive to metal ions. Consequently, do not allow any metal element to come into contact with the various conjugate or substrate solutions.
- Use a new distribution tip for each sample.
- Well washing is a critical step in this procedure: respect the recommended number of washing cycles and make sure that all wells are completely filled and then completely emptied. Incorrect washing may lead to inaccurate results.
- Never use the same container to distribute Conjugate and other solutions.
- Check the pipettes and other equipment for accuracy and correct operation.
- Do not change the assay's procedure.
- Use distilled or deionized water.
- Avoid exposure of the reagents to excessive heat or sunlight during storage and incubation.
- Once the assay has been started, all subsequent steps should be performed without interruption.

IV. HEALTH AND SAFETY INSTRUCTIONS

- All reagents included in the kit are intended for "*in vitro* diagnostic use".
- Human origin material used in the preparation of the Negative Control has been tested and found non-reactive for Hepatitis B surface antigen (HBsAg), antigen p24 HIV-1, antibodies to Hepatitis C virus and antibodies to human immunodeficiency virus (HIV-1 and HIV-2).
- Human origin material used in the preparation of the Positive Control has been tested and found non-reactive for Hepatitis B surface antigen (HBsAg), antigen p24 HIV-1 and antibodies to HIV-1,2.
- Because no known test method can offer complete assurance that infections agents are absent, handle reagents and patient samples as if capable of transmitting infections disease.
- Do not eat, drink, smoke, or apply cosmetics where immunodiagnostic materials are being handled.
- Any equipment directly in contact with samples and reagents as well as washing solutions should be considered as contaminated products and treated as such.
- Wear lab coats and disposable gloves when handling reagents and samples and thoroughly wash your hands after handling them.
- Avoid spilling samples or solutions containing samples. Wipe spills immediately and decontaminate affected surfaces.
- Avoid any contact of the Substrate Buffer, TMB, TMB-Substrate and the Stopping Reagent with the skin and mucosa.
- Provide adequate ventilation.
- All materials contacted with specimens or reagents, including liquid and solid wastes, should be inactivated by validated procedures (autoclaving or chemical treatment) and disposed in accordance with applicable local law regulations.

Warning!

Conjugate, Sample Diluent contain ProClin 300.
H317: May cause an allergic skin reaction.
P261: Avoid breathing vapors.
P280: Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352: IF ON SKIN: Wash with plenty of water.
P333 + P313 If skin irritation or rash occurs: Get medical advice/ attention.

Warning!

Positive Control, Negative Control contain sodium azide.
H312: Harmful in contact with skin.
P280: Wear protective gloves/protective clothing/ eye protection/face protection.
P302+P352 IF ON SKIN: Wash with plenty of water. Immediately call a poison center/doctor.
P312: Call a POISON CENTER or doctor/physician if you feel unwell.

Danger!

Stopping Reagent contains 0.2M sulfuric acid.
H314 Causes severe skin burns and eye damage.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P303 + P361 + P353 IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.
P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310 Immediately call a POISON CENTER or doctor/ physician.

V. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED WITH THE TEST

- Distilled or deionized water.
- Automatic or semiautomatic, adjustable or preset single-channel and multi-channel pipettes with a changeable volume for a set of liquids.
- Disposable pipette tips.
- Microplate incubator or thermoshaker at (37.0 ± 1.0) °C.
- Automatic microplate washer.
- Microplate reader equipped with 450 nm or with 450 and 620-680 nm filters.
- Laboratory clock.
- For automated EIA procedure – any model of open type automated EIA-analyzer.

VI. COLLECTION AND HANDLING OF SAMPLES

Blood samples should be collected according to the current practices. Human blood serum (plasma), immunoglobulins and other human serum (plasma) derived products may be used as samples. To exclude false positives, do not heat inactivate samples; select and store samples under the conditions preventing bacterial growth.

Store/transport samples in compliance with the current relevant regulations. Separate serum from red cells or plasma from the clot as soon as possible, to avoid haemolysis. Haemolysis can affect the assay performance. Do not analyze specimens with expressed bacterial growth, haemolysis, hyperlipidemia as it can yield incorrect results. Samples containing aggregates or sediment should be clarified by centrifugation.

Dilute lyophilized blood products according to IFU before testing. Liquid blood products can be used undiluted, except for immunoglobulin-based products. Immunoglobulin-based product should be preliminary diluted 50 times with Working Washing Solution.

VII. PREPARATION OF THE REAGENTS

1. Ready for use reagents:

- **HCV Ag Coated Plate.** Each 12-strips plate (breakable wells) is wrapped in a sealed foil-lined bag. Open the bag and remove the plate. Select the number of strips/wells required for the assay. Place the unused strips/wells back into the foil-lined bag; reseal the foil-lined bag in a Zip-Lock plastic bag. Do not remove desiccant.
- **Negative Control;**
- **Positive Control;**
- **Conjugate;**
- **Sample Diluent;**
- **Stopping Reagent;**
- **TMB-Substrate** (if TMB-Substrate is included into the kit).

2. Reagents to prepare:

- **Working Washing Solution.** Thoroughly mix the contents of the vial with concentrated Washing Solution (concentrated 25-fold). Dilute the required volume of concentrated Washing Solution with corresponding volume of distilled or deionized water (See Tables 2 and 3). Mix thoroughly the solution
- **Substrate Mixture.** Dilute the required volume of concentrated TMB (concentrated 11-fold) with the corresponding volume of Substrate Buffer (See Tables 2 and 3). Mix thoroughly.

Table 2

Consumption of reagents for the manual test procedure

Number of strips to be used	Working Washing Solution		Substrate Mixture	
	Washing Solution (concentrated 25-fold) (ml)	Distilled or deionized water (ml)	TMB (concentrated 11-fold) (ml)	Substrate Buffer (ml)
1	3.0	72.0	0.1	1.0
2	6.0	144.0	0.2	2.0
3	9.0	216.0	0.3	3.0
4	12.0	288.0	0.4	4.0
5	15.0	360.0	0.5	5.0
6	18.0	432.0	0.6	6.0
7	21.0	504.0	0.7	7.0
8	24.0	576.0	0.8	8.0
9	27.0	648.0	0.9	9.0
10	30.0	720.0	1.0	10.0
11	33.0	792.0	1.1	11.0
12 (a plate)	40.0	960.0	1.2	12.0

Table 3

Consumption of reagents for the automated test procedure

Number of strips to be used	Working Washing Solution (ml)		Substrate Mixture	
	Washing Solution (concentrated 25-fold) (ml)	Distilled or deionized water (ml)	TMB (concentrated 11-fold) (ml)	Substrate Buffer (ml)
4 (for Format 1)	16.0	384.0	0.65	6.5
8 (for Format 1)	32.0	768.0	1.2	12.0
12 (a plate)	40.0	960.0	1.2	12.0

VIII. TEST PROCEDURE

Note: Before use, allow reagents to reach room temperature (18-24 °C) for 30 min.

Attention! Incubation is possible as two alternative procedures. It is very important that the following assay step should be carried out in the same incubation mode. It is not allowed to combine the incubation modes.

Step	**Procedure 1** (thermoshaker)	**Procedure 2** (microplate incubator)
1	Add 60 µl of Sample Diluent into each well.	
2	Depending on the quantity of strips used in the assay, dispensing 40 µl of controls per well as described below is recommended: 1 strip – Positive Control to 1 well, Negative Control to 2 wells; 2 strips – Positive Control to 2 wells, Negative Control to 2 wells; 3 strips and more – Positive Control to 2 wells, Negative Control to 3 wells. The particular sequence and positions of control samples on the plate may be changed depending on the applicable automated system.	
3	Add 40 µl of undiluted samples into the rest of the wells. If the microplate incubator is used (procedure 2), mix the content of the wells by careful tapping on the edge of the plate. Pink color in wells should change to yellow when samples are added. The exceptions are individual samples with a pH close to the neutral value, which do not change the colour of Sample Diluent.	
4	Incubate the plate for **30 min** in shaker at 500 rpm at (37.0 ± 1.0) °C.	Incubate the plate for **60 min** in microplate incubator at (37.0 ± 1.0) °C.
5	Remove the content of the wells into the container with disinfectant solution. Add into each well not less than 400 µl of Working Washing Solution. Allow a soak time at least 40 seconds and aspire. Repeat this procedure three times (i.e. in total of four washes). Use of an automatic microplate washer is strongly recommended. Incomplete washing will adversely affect assay precision.	
6	Add 100 µl of Conjugate into each well.	
7	Incubate the plate for **20 min** in shaker at 500 rpm at (37.0 ± 1.0) °C.	Incubate plate for **20 min** in microplate incubator at (37.0 ± 1.0) °C.
8	Wash the plate 4 times as described in p. 5.	
9	Add 100 µl of Substrate Mixture or TMB-Substrate into each well.	
10	Keep plates in a dark place for 20 min at 18-24 °C or for 15 min at (37.0 ± 1.0) °C in a dark place.	
11	Add 150 µl of Stopping Reagent into each well and read the optical density at 450/620-680 nm using a plate reader at least 2-3 minutes after Stopping Reagent addition. Reading the absorbance at 450 nm only is possible.	

Scheme of the assay is provided in Annex.

Automated procedure with open automated analyzers TECAN Freedom EVOlyzer, TECAN, Switzerland (test procedure with other models of open automated analyzers is admissible).

1. Set the EIA protocol and start the analyzer.
2. Fill Working Washing Solution prepared in the designated container, place the remaining working solutions and reagents in special containers, Positive Control, Negative Control should be filled in vials, samples – in vials or tubes in an amount of not less than 300 µl, in the appropriate analyzer racks. Place the required number of plates in the analyzer. Then run the test procedure in accordance with the operator's manual for the analyzer and EIA protocol.
3. When the assay is finished, the analyzer generates results protocol which specifies characteristics of each sample and Positive Control and Negative Control.
4. Perform reading and interpretation of results as specified in section **IX**.

Spectrophotometric verification of samples and reagent dispensing when using the kit abia HCV Ab express on automated EIA-analyzers:

1. The addition of samples into the wells should be verified at 450 nm and 620 nm, criterion: OD > 0.180.
2. The addition of Conjugate should be verified at 450 nm and 620 nm, criterion: OD > 0.350.
3. The addition of Substrate Mixture should be verified at 540 nm, criterion: OD > 0.040.

IX. RESULTS

Assess the results spectrophotometrically at two wavelengths – 450 nm and at reference wavelength of between 620 and 680 nm with the air photometer adjustment. It is acceptable to read results at one wavelength – 450 nm.

To determine the presence of anti-HCV, compare optical density (OD) measured for each sample with the calculated Cut-Off value.

Consider test results if OD mean of Negative Control does not exceed 0.2*, OD value in the wells with Positive Control is not less than 1.5.

Calculate Cut-Off value as:

Cut-Off = average OD value of Negative Control + 0.300,

where **0.300** is a coefficient defined by manufacturer during statistical processing for each lot.

*****OD value of Negative Control and samples lower than 0.00 (with the mark "-") when calculating Cut-Off and results are considered as equal to zero.

Positive: if the OD value is ≥ Cut-Off.
Negative: if the OD value is < Cut-Off.

Reactive samples should be re-tested with this assay in at least two wells. If at least one of the repeated assays gives positive results, the sample is considered positive. If the results of re-testing are negative, the sample is considered negative. All positive samples should be tested in confirmatory EIA-tests, in immunoblotting or PCR.

X. PERFORMANCES

Diagnostic sensitivity

Diagnostic sensitivity of the abia HCV Ab express kit was evaluated when testing:

1. 439 serum samples from HCV patients. All samples positive for anti-HCV were tested positive. Sensitivity (95% CI): 439/439=100% (99.13% - 100%).

2. 14 commercial seroconversion panels (BBI, Inc.; Zeptometrix, USA). Results obtained are provided in Table 4 (it shows the number of samples in the panel tested positive out of the total number of samples of the seroconversion panel).

3. Standard panel of samples positive and negative for antibodies to Hepatitis C virus, "DS-STANDARD PANEL-ANTI-HCV" (RPC "Diagnostic Systems", Russia) and equivalent. Sensitivity of the kit with the panels was 100%.

All positive samples were confirmed using "DS-EIA-ANTI-HCV-SPECTRUM-GM" (RPC "Diagnostic Systems", Russia).

Table 4

Diagnostic sensitivity of the kit when testing seroconversion panels

No.	Panel	Number of positives as compared with the number of tested samples		
		abia HCV Ab express	Monolisa Anti-HCV Plus[1]	Abbott AxSYM HCV v.3.0 [2]
1	BBI PHV 906	5/7	5/7	Not tested
2	BBI PHV 911	3/4*	2/4*	Not tested
3	ZMC HCV 9046	4/5	4/5	Not tested
4	ZMC HCV 9047	4/10	3/10	Not tested
5	ZMC HCV 10057	2/7	2/7	Not tested
6	ZMC HCV 10058	7/7	7/7	Not tested
7	ZMC HCV 10062	2/8	2/8	Not tested
8	ZMC HCV 10071	6/7	4/7	Not tested
9	ZMC HCV 10185	4/5	4/5	Not tested
		37/60	**33/60**	-
10	ZMC HCV 6222	1/8	Not tested	1/8
11	ZMC HCV 6227	2/7	Not tested	2/7
12	ZMC HCV 9041	4/8	Not tested	4/8
13	ZMC HCV 9044	2/6	Not tested	2/6
14	ZMC HCV 9058	1/5	Not tested	0/5
Total		**10/34**	**-**	**9/34**

* There is no sample 1 in the panel.

[1] Results of RPC "Diagnostic Systems"

[2] Results of LS Medizin Service, April 2007

Specificity

Specificity of the abia HCV Ab express kit was evaluated when testing donor serum samples (n=5031). Specificity (95% CI): 5014/5031=99.66% (99.46% - 99.79%).

Additionally, 579 serum samples were tested:

- 372 samples of pregnant women and patients with infectious diseases;
- 207 serum samples of clinical patients with various non-infectious diseases.

Specificity with this selection (95% CI): 576/579=99.48% (98.49% - 99.82%). Specificity of the kit, evaluated with the Standard panel of samples positive and negative for antibodies to Hepatitis C virus, "DS-STANDARD PANEL-ANTI-HCV" (RPC "Diagnostic Systems", Russia) and its equivalent, was 100%.

The results are provided in Table 5.

Table 5

Sample category	Quality of samples	Quality of repeatedly reactive samples	Specificity	CI (95% CI)
Healthy donors	5031	17	99.66%	99.46% - 99.79%
Pregnant women and patients with infectious diseases	372	1	99.73%	98.49% - 99.95%
Clinical patients with various non-infectious diseases	207	2	99.03%	96.58% - 99.74%
Total	5610	20	99.64%	99.45% - 99.77%

Precision

Intra-plate reproducibility was assessed when testing 3 positive samples in 25 repeats with the kits of the same lot. The coefficient of variation did not exceed 8%.

Between-plate reproducibility was assessed when testing 3 positive samples in 25 repeats with the kits of the same lot. The coefficient of variation did not exceed 10%.

Inter-assay reproducibility was assessed when testing 3 positive samples in 25 repeats with kits of three lots. The coefficient of variation did not exceed 12%.

XI. LIMITS OF THE TEST

- Carefully read and strictly follow the instructions for use, especially stages of dispensing samples and reagents, plate washing and time of incubation.
- Use only undiluted serum or plasma samples for the assay.
- The interpretation of sample reactivity to viral Hepatitis C should not be based on a single reactive result. All positive samples should be verified with the confirmatory assays (EIA, immunoblotting or PCR).
- Negative results can occur if the quantity of antibodies to Hepatitis C virus present in the sample is too low for the detection of the kit, or if the marker to be detected is not present during the stage of disease in which the sample was collected. Negative screening test result does not exclude HCV infection.

- Differences in the results of samples from HCV patients with different assays can be caused by differences in immunological responses depending on the type of antigens used.
- The variability of HCV virus does not allow excluding the possibility of false negative results. No known test method can offer complete assurance that the HCV virus is absent.

XII. CONDITIONS OF STORAGE AND TRANSPORTATION

- **Expiry date is indicated on the packaging.** Storage and transportation conditions for the kit, conditions and terms of storage for working solutions and unused reagents are specified in Table 6.
- Transportation should be done at specified temperature in accordance with established transportation regulations. Kits transported at improper temperature cannot be used.
- Kits stored improperly cannot be used.

Table 6

1	**Storage conditions**		
	Keep in a dark dry place at 2-8 °C. Freezing is prohibited.		
2	**Transportation conditions**		
	at 2-8 °C		
	at 9-20 °C	not more than during ten (10) days	
3	**Conditions and terms of storage for working solutions** Keep in a dark dry place and in a chemically neutral vial		
	Working Washing Solution	at 2-8 °C	For up to 28 days
		at 18-24 °C	For up to 14 days
	Substrate Mixture	at 18-24 °C	For up to 10 hours
4	**Conditions and terms of storage of unused reagents after opening**		
	Keep in a dark dry place at 2-8 °C.		
	HCV Ag Coated Plate	Place the unused strips/wells back into the bag, reseal the foil-lined package in Zip-Lock polyethylene bag. Do not remove desiccant.	Until the kit expiration date
	Positive Control, Negative Control, Conjugate, Sample Diluent, Washing Solution, Substrate Buffer, TMB, TMB-Substrate, Stopping Reagent	Close the vials tightly with screw caps and store them in the manufacturer's package.	Until the kit expiration date

XIII. GUARANTEE

- Manufacturer guarantees conformity of the product to the requirements of regulatory and technical documentation.
- Quality and safety of the kit is guaranteed within established shelf life.
- Please contact the Manufacturer if you have any questions.

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XIV. REFERENCES

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7. Bochkova G.B., Fomina S., Puzyrev V.F., Obriadina A.P., Burkov A.N., Ulanova. T.I. The evaluation of the ELISA kit "DS-EIA-ANTI-HCV-SPECTRUM-GM" as supplemental assay for confirmation of anti-HCV screening positive results // 21-th European Congress of Clinical Microbiology and Infection Diseases. – Milan, May 7-10, 2011. – P.2235.

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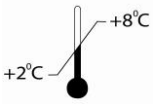
XV. EXPLANATION OF SYMBOLS

	Manufacturer		Storage temperature limitation
	Date of manufacture CCYY-MM		Consult Instruction for use
	Expiry date CCYY-MM-DD		For *in vitro* diagnostic use
	Batch code		Sufficient for
	Catalog number		Symbol “exclamation mark”
	Fragile, handle with care	**Warning!**	Signal word
	Keep away from sunlight		Symbol “corrosion”
	Keep dry	**Danger!**	Signal word
	Top		

Annex

Scheme of the assay

1	**Add**	60 µl of Sample Diluent
2	**Add**	40 µl of Positive Control, Negative Control
3	**Add**	40 µl of samples
4	**Incubate**	**Procedure 1:** 30 min at (37.0 ± 1.0) °C, 500 rpm, thermoshaker **Procedure 2:** 60 min at (37.0 ± 1.0) °C, microplate incubator
5	**Wash the plate**	**Working Washing Solution,** 4 times, not less than 400 µl
6	**Add**	100 µl of Conjugate
7	**Incubate**	**Procedure 1:** 20 min at (37.0 ± 1.0) °C, 500 rpm, thermoshaker **Procedure 2:** 20 min at (37.0 ± 1.0) °C, microplate incubator
8	**Wash the plate**	**Working Washing Solution,** 4 times, not less than 400 µl
9	**Add**	100 µl of Substrate Mixture or TMB-Substrate
10	**Incubate**	20 min at 18-24 °C or 15 min at (37.0 ± 1.0) °C in a dark place
11	**Add**	150 µl of Stopping Reagent
12	**Read the optical density**	450 nm/620-680 nm or 450 nm