AB Diagnostic Systems

REF **DK.055.01.8** Σ **96** IVD **For *In vitro* Diagnostic Use**

INSTRUCTIONS FOR USE
abia Toxo IgG
Enzyme immunoassay for the qualitative and quantitative determination of IgG antibodies to *Toxoplasma gondii* in human serum or plasma

This Package Insert provides information for Professional Use of the kit.
The kit contains sufficient reagents for 96 (one breakable plate) assays including controls; the kit is intended for manual testing with a possibility of fractional (one strip) use of the kit or for use of the kit on open type automated analyzer for enzyme immunoassay.

I. INTENDED USE

The abia Toxo IgG kit is intended for the qualitative and quantitative determination of IgG antibodies to *Toxoplasma gondii* in human serum (plasma) by a microplate immunoenzymometric assay.

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. INTRODUCTION

T. gondii is an obligate intracellular protozoan parasite with a worldwide distribution [1, 2]. Serological data indicate that approximately 30% of the population of most industrialized nations is chronically infected with the organism [3]. When a seronegative woman becomes infected *T. gondii* during pregnancy, the organism is often transmitted across the placenta to the fetus [1, 4]. The severity of infection in the fetus varies with the trimester during which the infection was acquired. Infection during the trimester may lead to spontaneous abortion, stillbirth or overt disease in the neonate. Approximately 75% of congenitally infected newborns are symptomatic. However, nearly all children born with subclinical toxoplasmosis will develop adverse ocular or neurologic sequelae later in life [4, 5]. Approximately 80-85% develops chorioretinitis and some may also experience blindness or mental retardation.

III. PRINCIPLE OF THE TEST

Scheme of the test procedure is an indirect two-stage immunoassay. Microtiter strip wells are coated with the recombinant antigens of *Toxoplasma gondii* to bind corresponding antibodies. The antigen-antibody complex reacted with HRP-labeled anti-human-IgG antibodies. The presence of bound enzyme indicating the presence in the specimen of specific antibodies is revealed by a color change in the TMB-Substrate. The color development is stopped with the addition of Stopping Reagent, changing the color to yellow. The color intensity of the test sample is directly proportional to the concentration of IgG antibodies specific to *Toxoplasma gondii*.

IV. CONTENT OF THE KIT abia Toxo IgG

4.1 Contents of the reagent kit.

Table 1

LABEL	NATURE OF THE REAGENTS	PRESENTATION
T. gondii-Ag Coated Strips	Polystyrene stripped 96-well plate (breakable wells) coated with mix of recombinant analogs of *T. gondii* antigens. Store at 2-8 °C until expiration date.	1 plate
Conjugate	Antibodies against human IgG, conjugated with HRP enzyme with addition of 1M Tris HCl buffer (pH 7.4-7.6), Tween®20 (0.11%) and bovine serum albumin (3.85%). Preserving agents: 0.10% ProClin 300, 0.01% phenol, 0.004% gentamicin sulfate. Transparent or slightly opalescent light yellow liquid. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 11.0 ml
Positive Control, Inactivated	Control sample, containing IgG antibodies to *T. gondii.* Preserving agents: 0.04% ProClin 300, 0.20% sodium azide. Transparent or slightly opalescent red colored liquid. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 1.2 ml
Negative Control, Inactivated	Control sample, not containing IgG antibodies to *T. gondii.* Preserving agents: 0.04% ProClin 300, 0.20% sodium azide, 0.001% gentamicin sulfate. Transparent or slightly opalescent green colored liquid. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 2.5 ml
Calibrator, Inactivated	Human serum containing IgG antibodies to *T. gondii* with addition of bovine serum albumin (4.88%)*. Preserving agents: 0.10% sodium azide, 0.10% phenol. Transparent or slightly opalescent light yellow liquid. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 2.5 ml

Preliminary Sample Diluent	Sample buffer that is used for preliminary dilution of samples. Preserving agent: 0.09% sodium azide. Transparent or slightly opalescent violet-blue colored liquid. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 11.0 ml
Sample Diluent	Sample buffer that is used to dilute samples. Preserving agents: 0.10% sodium azide, 0.006% thimerosal. Transparent or slightly opalescent pink colored liquid. Sediment may form. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 11.0 ml
Washing Solution (concentrated 25-fold)	Phosphate-saline solution (pH 7.4-7.7). Transparent or slightly opalescent colorless or light yellow liquid, sediment may form that dissolves completely at 35-39 °C and shaking. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 50.0 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
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
Plate for preliminary dilution of sera	Polystyrene plate with transparent wells.	1 plate
Protective films for EIA plates		2
Polyethylene bag with a Zip-Lock		1
Disposable plastic dishes for liquid reagents		2
Disposable tips		16

* Exact level is given in Table 3.

V. 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. If labels are lost or labels/vials damage, vials should be disposed, and **kit cannot be used**.
- Do not use expired reagents.
- Do not mix reagents from different lots within a given test run.
- 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.
- 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.
- Check the pipettes and other equipment for accuracy and correct operation.
- Do not change the assay's procedure.
- Use high quality water.
- Avoid exposure of the reagents to excessive heat or sunlight during storage and incubation.

VI. 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 Positive Control, Negative Control and Calibrator has been tested and found negative for HBsAg, antibodies to hepatitis C virus and antibodies to human immunodeficiency virus (HIV-1 and HIV-2), antigen p24 HIV-1.
- Because no known test method can offer complete assurance that infectious agents are absent, handle reagents and patients samples as if capable of transmitting infectious disease.
- Do not eat, drink, smoke, or apply cosmetics where immunodiagnostic materials are being handled.
- Any equipment directly in contact with specimens 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 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 contains ProClin 300.
H317: May cause an allergic skin reaction.
P261: Avoid breathing vapours.
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.

Danger!

Positive Control, Negative Control, Calibrator, Sample Diluent 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 soap and water.
P312: Call a POISON CENTER or doctor/physician if you feel unwell.

Danger!

Stopping Reagent contains 0.2 M/l sulphuric 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.

VII. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED WITH THE KIT:

- 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 at (37.0 ± 1.0) °C.
- Automatic microplate washer.
- Microplate reader equipped with 450 nm or with 450 and 620-680 nm filters;
- Open type automated analyzer with 450 nm or with 450 and 620-680 nm filters (for automated procedure);
- Laboratory clock.

VIII. COLLECTION AND HANDLING OF SPECIMENS

Collection of blood samples should be implemented according to the current practices. Serum, plasma (citrate, heparin, EDTA) may be used. Separate serum or plasma from blood cells as soon as possible to avoid any hemolysis. Extensive hemolysis may affect test performance. Specimens with observable particulate matter should be clarified by centrifugation prior to testing. Suspended fibrin particles or aggregates may yield falsely positive results.

Samples can be stored at 2-8 °C no longer than eight days. For long-term storage separated serum/plasma should be frozen at or below -20 °C. Samples that have been frozen and defrosted more than 1 time cannot be used.

IX. PREPARATION OF THE REAGENTS

1. Ready to use reagents:

- **T. gondii-Ag Coated Strips.** Strips are wrapped in a sealed foil-lined bag. Open the bag and remove the tray. Select the number of Coated Strips required for the assay. Return unused strips in the bag. After the bag has been opened the Coated Strips are stable during the shelf life of the kit at 2-8 °C, provided that the foil-lined bag is resealed in Zip-Locked plastic bag. The silica gel bag should not be removed from the foil packaging.
- **Conjugate;**
- **Positive Control;**
- **Negative Control;**
- **Calibrator;**
- **Preliminary Sample Diluent;**
- **Sample Diluent;**
- **Stopping Reagent;**
- **TMB-Substrate.**

2. Reagents to prepare:

- **Working Washing Solution**. Thoroughly shake Washing Solution concentrate. To make Working Washing Solution take required amount of concentrate and mix with distilled or deionized water (1:24 ratio) in a separate vial. Thoroughly mix the solution. The prepared Working Washing Solution is stable for 14 days at 18-24 °C or for 28 days at 2-8 °C.

The required volumes of Working Washing Solution for the certain number of strips or plate are tabulated in Table 2.

Table 2

Number of strips to be used		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**1 well**
Working Washing Solution	Washing Solution (×25), ml	3.0	6.0	9.0	12.0	15.0	18.0	21.0	24.0	27.0	30.0	33.0	40.0	0.2
	High quality water, ml	72.0	144.0	216.0	288.0	360.0	432.0	504.0	576.0	648.0	720.0	792.0	960.0	4.8

3. Storage of unused reagents

After opening the vials the unused components of the kit: Positive Control, Negative Control, Calibrator, Preliminary Sample Diluent, Sample Diluent, Washing Solution (concentrated 25-fold), Stopping Reagent can be stored in tightly sealed vials until the kit expiration date at 2-8 °C. Coated Strips can be stored until the kit expiration date at 2-8 °C.

Conjugate, TMB-Substrate can be stored in tightly sealed vials within 3 months at 2-8 °C.

X. TEST PROCEDURE

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

Step	The assay procedure
1	Add 90 µl of Preliminary Sample Diluent into the wells of the plate for preliminary samples dilution and 10 µl of the tested serum (plasma) samples. Carefully mix by pipetting. Violet-blue color should change to blue-green.
2	Add 100 µl of Positive Control, Negative Control and Calibrator into the wells. 1 strip – Positive Control to 1 well, Negative Control to 1 well and Calibrator to 2 wells; 2 strips and more – Positive Control to 1 well, Negative Control to 2 wells and Calibrator to 3 wells.
3	Add 90 µl of Sample Diluent and 10 µl of the preliminary diluted samples to the rest of the wells (the final serum dilution ratio is 1:100). Carefully mix fluid in wells by gentle pipetting. Cover the strips with a protective film.
4	Incubate for **30 min** in a microplate incubator at (37.0 ± 1.0) °C.
5	Aspirate the contents of the wells and wash the plate **4 times** with the Working Washing Solution. Add into each well not less than 380 µl of Working Washing Solution and remove Washing Solution into the container with disinfecting solution. Do not leave any fluid in the wells. Use of an automatic microplate washer is strongly recommended. Incomplete washing will adversely affect the assay precision.
6	Add 100 µl of Conjugate to all the wells of the plate. Cover the plate with a protective film.
7	Incubate for **30 min** in a microplate incubator at (37.0 ± 1.0) °C. Remove fluid from wells, wash the plate **4 times** as described in step 5.
8	Add 100 µl of TMB-Substrate into all the wells.
9	Incubate at 18-24 °C for a **20 min** in a dark place.
10	Add 150 µl of Stopping Reagent into wells to stop the reaction results are read by microplate plate reader at wavelength of 450 nm, with reference filter at 620-680 nm. Reading of the absorbance at 450 nm only is possible.

Scheme of the assay is represented in Annex.

Automated analyzer

Validated test protocols and dilution tables of reagent working solutions for different EIA-analyzers can be obtained from the manufacturer upon request (see section XIV). For the instrumentation without established validated protocol follow the section "TEST PROCEDURE" and ensure all requirements described in the section "PRECAUTIONS" are fulfilled. All protocols for automated analyzers must be fully validated before use.

When preparing working reagent solutions for automated EIA procedure, it is necessary to consider "dead" volume of vials and containers used for loading working solutions in the EIA analyzer.

XI. RESULTS

Test Validation

For the test to be valid the following criteria must be met. If these criteria are not met the test should be considered invalid and should be repeated.

1. **Positive Control:** The absorbance value should not be less than 1.3.
2. **Negative Control:** The absorbance value should not be more than 0.200.
3. **Calibrator:** The absorbance value should not be less than 0.25.

Qualitative results

The presence or absence of antibodies against *Toxoplasma gondii* is determined by the ratio of the OD of each sample to the calculated Cut-Off value.

Calculate Cut-Off value as:

Cut-Off = average OD value of Calibrator / A (A = 4.2) (1),

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

Interpretation of Result

Sample is positive, if the OD value is ≥ Cut-Off.
Sample is negative, if the OD value is < Cut-Off.

Quantitative results

The concentration of ANTI-TOXO-IgG calculates only for positive samples.

Concentration [IU/ml] = OD sample x B / average OD value of Calibrator (2)

B – anti-TOXO IgG concentration of the Calibrator (IU/ml) (Table 3).

Table 3

Concentration of IgG antibodies specific to *Toxoplasma gondii* in Calibrator

Reference material, used for certification of the Calibrator	Concentration of IgG antibodies specific to *Toxoplasma gondii* in Calibrator, IU/ml	Interpretation of results
«The 3rd International Standard for ANTI-TOXOPLASMA SERUM, HUMAN», TOXM (lot 003), NIBSC code, United Kingdom	125.0	≥ 30 IU/ml, Positive < 30 IU/ml, Negative
WHO International Standard Anti-Toxoplasma IgG, Human, NIBSC code: 01/600, NIBSC, United Kingdom	5.0	≥ 1.2 IU/ml, Positive < 1.2 IU/ml, Negative

If in an initial assay the OD of tested specimen exceeds 2.000, the specimen must be diluted 10 times or more with a Working Washing Solution and reassayed as described in TEST Procedure. For the calculation of the concentrations this dilution factor has to be taken into account.

XII. PERFORMANCE CHARACTERISTICS

1. Analytical sensitivity

The analytical sensitivity was calculated by adding 2 standard deviations from the mean of 20 replicate analyses of Negative Control and was found to be 1.656 IU/ml according to The 3rd International Standard for ANTI-TOXOPLASMA SERUM, HUMAN», TOXM (lot 003), NIBSC, United Kingdom and 0.066 IU/ml according to WHO International Standard Anti-Toxoplasma IgG, Human, NIBSC: 01/600, NIBSC, United Kingdom.

2. Interferences

Hemoglobin (up to 10 mg/ml), bilirubin (up to 0.2 mg/ml) and lipids (up to 20 mg/ml) have no influence on the assay results.

3. Cross reactivity

No cross reactivity was found for samples with antibody to Epstein-Barr virus.

4. Diagnostic sensitivity

Diagnostic sensitivity of abia Toxo IgG with 144 anti-TOXO IgG positive samples is 100% (95%CI: 97.4-100%).

5. Diagnostic specificity

Diagnostic specificity of abia Toxo IgG with 103 anti-TOXO IgG negative samples is 99.0 % (95% CI: 94.7-99.8).

6. Trueness. Agreement with certified reference measurement procedure

The abia Toxo IgG was compared with the "Toxoplasma IgG" (BCM Diagnostics, USA). 270 serum and plasma samples were tested.

	Toxoplasma IgG (BCM Diagnostics, USA)			
abia Toxo IgG		Positive	Negative	Indeterminate
	Positive	166	2	4
	Negative	0	84	14

The abia Toxo IgG has not a "gray area", so indeterminate results were not included in the calculation. The agreement to comparative assays is 99.2% (95%CI: 97.2-99.8%).

7. Precision

The precision of the abia Toxo IgG was determined by 20 days × 3 samples × 2 replicates covering the measuring range.

Intra-assay (within run) precision

Data	Serum sample #1	Serum sample #2	Serum sample #3
Mean (IU/ml)	126.2	63.4	32.9
Sr	3.92	1.71	2.32
CV (%)	3.1	2.7	7.0

Inter-assay (between-run) precision

Data	Serum sample #1	Serum sample #2	Serum sample #3
Mean (IU/ml)	126.2	63.4	32.9
S_{rr}	5.80	0.51	1.21
CV (%)	4.6	0.8	3.7

8. Recovery

Concentration of IgG specific to *Toxoplasma gondii* in Calibrator of the kit abia Toxo IgG - 125 IU/ml (according to WHO International Standard Anti-Toxoplasma Serum Ig NIBSC code: TOXM international standard).

The results are tabulated below:

Sample	Measured concentration, IU/ml	Expected concentration, IU/ml	Recovery, %
Control serum	82.5	-	-
Calibrator	125.0	-	-
Control serum + Calibrator	94.4	103.8	91.0

Concentration of IgG specific to *Toxoplasma gondii* in Calibrator of the kit abia Toxo IgG - 5 IU / ml (according to WHO International Standard Anti-Toxoplasma IgG NIBSC code: 01/600).

Sample	Measured concentration, IU/ml	Expected concentration, IU/ml	Recovery, %
Control serum	3.3	-	-
Calibrator	5.0	-	-
Control serum + Calibrator	3.8	4.2	91.0

9. Linearity

The linearity of the test was investigated using serial dilutions of patient sera with high anti-TOXO IgG concentration. According to WHO International Standard Anti-Toxoplasma Serum Ig NIBSC code: TOXM international standard the abia Toxo IgG is linear in the measurement range from 62.3 to 170.4 IU/ml.

10. Hook effect

No hook effect was observed up to anti-TOXO IgG concentration of 344.1 IU/ml (according to WHO International Standard Anti-Toxoplasma Serum Ig NIBSC code: TOXM international standard) and 10.3 IU/ml (according to WHO International Standard Anti-Toxoplasma IgG NIBSC code: 01/600).

XIII. LIMITS OF THE TEST

1. A positive test result indicate that there has been contact with the pathogen at some undetermined time.

2. A negative serological result does not exclude an infection. Particularly in the early phase of an infection, antibodies may not be present or are only present in such small quantities that they are not detectable.

3. Demonstration of seroconversion from a negative to a positive titer or more than a fourfold increase in titer can indicate an acute infection when specimens drawn at least two weeks apart are tested within the same test. To investigate titer changes, sample and follow-up sample should be incubated in adjacent wells of the ELISA microplate within the same test run.

4. For diagnosis, the clinical picture of the patient always needs to be taken into accont along with the serological findings.

5. Immunocompetent women who have IgG antibody before conception are considered immune and so at very little risk for transmission of infection to the fetus. Women who are seronegative are considered at risk for infection.

6. Diagnosis of Toxoplasma infection in the newborn is made through a combination of serologic testing, parasite isolation and PCR [6].

XIV. CONDITIONS OF STORAGE AND TRANSPORTATION

Expiry date is indicated on the packaging.

Keep in dark dry place at 2-8 °C. Freezing is prohibited.

Transportation should be done at 2-8 °C. Transportation at 9-25 °C is allowed not more than during ten (10) days.

AB Diagnostic Systems GmbH
Sportfliegerstraße, 4, Berlin, 12487, Germany
Tel. +49 30 208987160, Fax: +49 30 208987199
E-Mail: info@ab-ds.de, www.ab-ds.de

XV. REFERENCES

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2. Anderson S.E. and Remington J.S.: The diagnosis of Toxoplasmosis. So. Med. J. 68:1433-1443, 1975.

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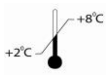
XVI. EXPLANATION OF SYMBOLS

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

Annex

Scheme of the assay

1	**Add**	90 µl of Preliminary Sample Diluent and 10 µl of the samples (conduct on the plate for preliminary dilution of samples)
2	**Add**	100 µl of Positive Control, Negative Control, Calibrator
3	**Add**	90 µl of Sample Diluent
4	**Add**	10 µl of preliminary diluted samples
5	**Incubate**	30 min, (37.0 ± 1.0) °C, microplate incubator
6	**Wash the plate**	**Working Washing Solution, not less than 380 µl, 4 times**
7	**Add**	100 µl of Conjugate
8	**Incubate**	30 min, (37.0 ± 1.0) °C, microplate incubator
9	**Wash the plate**	**Working Washing Solution, not less than 380 µl, 4 times**
10	**Add**	100 µl of TMB-Substrate
11	**Incubate**	20 min, 18-24 °C in a dark place
12	**Add**	150 µl of Stopping Reagent
13	**Read the optical density**	450 nm/620-680 nm or 450 nm