AB Diagnostic Systems

REF **DK.056.01.8** Σ **96** IVD **For *In vitro* Diagnostic Use**

INSTRUCTIONS FOR USE
abia Toxo IgA
Enzyme immunoassay for the detection of IgA 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 IgA kit is intended for the detection of IgA 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

Toxoplasma 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 *Toxoplasma 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-IgA antibodies. The presence of bound enzyme indicating the presence in the specimen of specific antibodies is revealed by a color change in the Substrate Mixture (TMB + Substrate Buffer). 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 IgA antibodies specific to *Toxoplasma gondii*.

IV. CONTENT OF THE KIT abia Toxo IgA

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 *Toxoplasma gondii* antigens. Store at 2-8 °C until expiration date.	1 plate
Conjugate (concentrated 11-fold)	Antibodies against human IgA, conjugated with HRP enzyme with addition of bovine serum albumin (1.6%) and Tween®20 (0.10%). Glycerol based solution. Preserving agents: 0.03% ProClin 300, 0.08% phenol, 0.0008% gentamicin sulfate. Transparent or slightly opalescent light yellow liquid. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 1.5 ml
Positive Control	Control sample, containing Anti-GST antibodies, conjugated with HRP enzyme with addition of Tween®20 (0.10%). Preserving agents: 0.10% ProClin 300. Transparent or slightly opalescent crimson red colored liquid. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 1.5 ml
Negative Control, Inactivated	Control sample, not containing IgA antibodies to *T. gondii*. Preserving agents: 0.04% ProClin 300, 0.19% 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 3.0 ml
Conjugate Diluent	Transparent yellow liquid at temperature of 2-8 °C, opalescence develops at temperature of 18-24 °C. Preserving agent: 0.01% thimerosal. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 13.5 ml
Preliminary Sample Diluent	Sample buffer that is used for preliminary dilution of samples. Preserving agent: 0.01% thimerosal. Transparent violet-blue colored liquid. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 12.5 ml
Sample Diluent	Sample buffer that is used to dilute samples. Preserving agents: 0.01% thimerosal. Transparent pale pink liquid. Sediment may form that dissolves completely at shaking causing the solution turbidity. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 12.5 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
Substrate Buffer	Citric acid (0.64%) solution, pH 4.1-4.3, containing H_2O_2 (0.008%). Transparent colorless liquid. Preserving agent: 0.04 % ProClin 300. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 15.0 ml
TMB (concentrated 21-fold)	Solution containing 3,3',5,5'-Tetramethylbenzidine (TMB) (0.26%), dimethyl sulfoxide (DMSO) (69.20%) and 1-Methyl-2-pyrrolidinone (6.90%). Transparent colorless liquid. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 1.5 ml

Stopping Reagent	Sulfuric acid solution (H_2SO_4) 0.75M. Transparent colorless liquid. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 25.0 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

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.
- 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.
- 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.
- Do not reuse protective films fo EIA plates.
- 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.

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 Negative Control 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.
- Certain reagents contain biological material of animal origin.
- 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 Substrate Buffer, TMB 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!

Positive Control contains 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!

Negative Control contains 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!

TMB contains N-Methyl-2-pyrrolidon:
H319: Causes serious eye irritation.
H360D: May damage the unborn child.
P264: Wash hands thoroughly after handling.
P280: Wear protective gloves/protective clothing/eye protection/face protection.
P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P308 + P313: IF exposed or concerned: Get medical advice/attention.

Danger!

Stopping Reagent contains 0.75M 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.

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 haemolysis. Extensive haemolysis 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.

Store/transport the samples in accordance with the current regulatory documentation. If samples are to be stored/transported for a longer period of time, they must be frozen at or below -20 °C. Avoid repeated freeze/thaw cycles. Samples that have been frozen and defrosted more than 1 time cannot be used. Samples with expressed haemolysis, hyperlipidemia must not be analyzed.

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 within 6 month after opening when stored 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.
- **Positive Control;**
- **Negative Control;**

- **Preliminary Sample Diluent;**
- **Sample Diluent;**
- **Stopping Reagent;**

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.
- **Working Solution of Conjugate.** Thoroughly mix the contents of the vial with concentrated Conjugate. For the working solution of Conjugate preparation, dilute the required volume of Conjugate (concentrated 11-fold) with the required volume of Conjugate Diluent (See Table 2) and mix thoroughly avoiding foaming. Store for not more than 12 hours at 18-24 °C in a dark place in clean vials.
- **Substrate Mixture.** Dilute the required volume of TMB (concentrated 21-fold) with the corresponding volume of Substrate Buffer (See Table 2). Mix thoroughly. It is possible to prepare Substrate Mixture directly in the vial with Substrate Buffer. For this purpose, pipette the required volume of TMB (concentrated 21-fold) (1.5 ml) in to the vial with Substrate Buffer (15.0 ml) and mix thoroughly. Store for not more than 10 hours at 18-24 °C in a dark place in clean vials.
  Substrate Mixture should be colorless!

The volume of reagents required for the certain number of strips or plate is provided in the table below:

Table 2

Reagent preparation

Number of strips to be used	Working Washing Solution		Working Solution of Conjugate		Substrate Mixture	
	Washing Solution (concentrated 25-fold) (ml)	Distilled or deionized water (ml)	Conjugate (concentrated 11-fold) (ml)	Conjugate diluent (ml)	Substrate Buffer (ml)	TMB (concentrated 21-fold) (ml)
1	4	96	0.1	1.0	1.0	0.05
2	8	192	0.2	2.0	2.0	0.10
3	12	288	0.3	3.0	3.0	0.15
4	16	384	0.4	4.0	4.0	0.20
5	20	480	0.5	5.0	5.0	0.25
6	24	576	0.6	6.0	6.0	0.30
7	28	672	0.7	7.0	7.0	0.35
8	32	768	0.8	8.0	8.0	0.40
9	36	864	0.9	9.0	9.0	0.45
10	40	960	1.0	10.0	10.0	0.50
11	44	1056	1.1	11.0	11.0	0.55
12	48	1152	1.2	12.0	12.0	0.60

3. Storage of unused reagents

After opening the vials the unused components of the kit: Conjugate (concentrated 11-fold), Positive Control, Negative Control, Conjugate Diluent, Preliminary Sample Diluent, Sample Diluent, Washing Solution (concentrated 25-fold), Substrate Buffer, TMB and Stopping Reagent can be stored in tightly sealed vials until the kit expiration date at 2-8 °C. Coated Strips are stable within 6 month after opening when stored 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	Wash the plate with Working Washing Solution twice before assay run. Carefully fill all wells with at least 380 µl of Working Washing Solution with multichannel pipette or automatic microplate washer, wait for 40 seconds and remove Working Washing Solution from the wells. 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.
2	Add 90 µl of Preliminary Sample Diluent into the wells of the plate for preliminary samples dilution and 10 µl of the samples. Carefully mix fluid in wells by gentle pipetting. Violet-blue color should change to green-blue. If you do not observe change of the color then there is no serum added to the well. Diluted samples do not store!
3	Add 100 µl of Positive Control, Negative Control into the wells of Coated Strips. 1 strip – Positive Control to 1 well and 2 wells with Negative Control; 2 strips – Positive Control to 2 wells and 2 wells with Negative Control; 3 strips or more – Positive Control to 2 wells and 3 wells with Negative Control.
4	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.
5	Cover the strips with a protective film. Incubate for **30 min** in a microplate incubator at (37.0 ± 1.0) °C.
6	Aspirate the contents of the wells into the container with disinfecting solution. Wash the plate **6 times** with the Working Washing Solution as described in step 1.
7	Add 100 µl of Working Solution of Conjugate into all wells.
8	Cover the plate with a protective film. Incubate for **30 min** in a microplate incubator at (37.0 ± 1.0) °C.
9	Aspirate the contents of the wells into the container with disinfecting solution. Wash the plate **6 times** with the Working Washing Solution as described in step 1.
10	Add 100 µl of Substrate Mixture into all the wells.
11	Incubate at 18-24 °C for a **20 min** in a dark place.
12	Add 50 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

For automated test procedure, it is advisable to use protocol submitted by the manufacturer. When creating the protocol independently, follow the procedure specified in section X TEST PROCEDURE, and comply with the requirements provided in sections V. PRECAUTIONS.

When preparing working solutions of reagents for the automated test procedure, dead volume of vials or containers used to place the solutions onboard should be taken into account.

Validated test protocols and dilution tables of working solutions for different models of EIA analyzers can be obtained upon request from the manufacturer (see section XV).

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 0.600.
2. **Negative Control:** the absorbance value should not be more than 0.150*.

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 Negative Control + A (A = 0.150),

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.

*calculating Cut-Off and evaluating the results, negative values of OD of Negative Control and tested specimens (with "-" sign) are deemed to be "zero".

XII. PERFORMANCE CHARACTERISTICS OF abia Toxo IgA

The kit sensitivity and specificity when studying clinical patient samples was 98% and 97%, respectively.

XIII. LIMITS OF THE TEST

1. A positive test result indicate exposure to the pathogen in the previous 12 months.

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

4. Toxo IgA becomes detectable from 14 days after infection, reaching its highest concentration after one month. Toxo IgA antibodies disappear after 6 months in about 90% of cases but may remain for more than 1 year.

5. Toxo IgA indicates primary infection within the past 12 months.

The detection of IgA antibodies is important for serological confirmation of the acute infection phase.

6. The IgA response can normally vary. There can be no response, but the presence of IgA antibodies can confirm the acute toxoplasmosis. The IgA response solely does not provide essential information for the acute infection phase.

7. IgA does not cross the placenta, therefore, the determination of Toxo IgA is used for the diagnosis of embryonic infection, neonatal and postnatal monitoring of congenital toxoplasmosis.

8. 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 9-20 °C is allowed not more than during ten (10) days.

XV. 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 Manufacturer if you have any questions.

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

XVI. REFERENCES

1. Krick J.A. and Remington J.S.: Toxoplasmosis in the adult: An overview. New Eng. J. Med. 298:550-553, 1978.

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

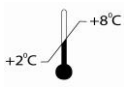
3. Feldman H.A.: Toxoplasmosis: An overview. Bull. N.Y. Acad. Med. 50:110-127, 1974.

4. Wilson C.B., Remington J.S., Stagno S. and Retnolds D.W.: Development of adverse sequelae in children born with subclinical congenital Toxoplasma infection. Pediatrics 66:767-774, 1980.

5. Berrebi A.; Kobuch W.E.; Bessieres M.H.; Bloom M.C.; Rolland M.; Sarramon M.F.; Roques C.; Fournie A.: Termination of pregnancy for maternal toxoplasmosis. 344 (8914) P. 36-9 Lancet (England), July 2 1994.

6. M36-A Vol.24, No.6: Clinical use and Interpretation of Serologic Tests for *Toxoplasma gondii*, Approved Guideline, 2004.

XVII. EXPLANATION OF SYMBOLS

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

Annex

Scheme of the assay

1	**Wash the plate**	Working Washing Solution, not less than 380 µl, 2 times
2	**Add**	90 µl of Preliminary Sample Diluent and 10 µl of the samples (conduct on the plate for preliminary dilution of samples)
3	**Add**	100 µl of Positive Control, Negative Control (wells of the Coated Strips) 1 strip – Positive Control to 1 well and 2 wells with Negative Control 2 strips – Positive Control to 2 wells and 2 wells with Negative Control 3 strips or more – Positive Control to 2 wells and 3 wells with Negative Control
4	**Add**	90 µl of Sample Diluent and 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, 6 times**
7	**Add**	100 µl of Working Solution 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, 6 times**
10	**Add**	100 µl of Substrate Mixture
11	**Incubate**	20 min, 18-24 °C in a dark place
12	**Add**	50 µl of Stopping Reagent
13	**Read the optical density**	450 nm/620-680 nm or 450 nm