AB Diagnostic Systems

REF **DK.058.01.8** Σ **96** IVD **For *In vitro* Diagnostic Use**

INSTRUCTIONS FOR USE
abia Toxo IgM Capture
Enzyme immunoassay for the detection of IgM 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 IgM Capture kit is intended for the detection of IgM antibodies to *Toxoplasma gondii* in human serum (plasma) by a microplate enzyme immunoassay.

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 intracellular protozoan parasite. The only known definitive hosts for *T. gondii* are domestic cats and other members of the family Felidae. Humans can become infected by any of several routes: eating undercooked meat of animals harboring tissue cysts, consuming food or water contaminated with cat feces or by contaminated environmental samples (such as contaminated soil or changing the litter box), blood transfusion or organ transplantation and transplacentally from mother to fetus [1].

Primary infection in a pregnant woman presents a particularly challenging issue for obstetricians and neonatologists due to the potential risk of transplacental transmission and fetal infection. The risk for transmission increases with gestational age while symptom intensity increases with duration of pregnancy. In the first trimester, the infection may result in miscarriage or intrauterine fetal death. In the other trimesters, it may lead to non-immune hydrops fetalis, hydrocephaly, microcephaly, intracranial calcification, or retinitis. Damage to the fetus may result in blindness, disturbed psychomotor and mental development, preterm labor, intrauterine growth restriction, and neonatal demise [2].

The ability to identify *T. gondii* infection is still today primarily based on serological assay detection of IgM, IgG, IgE and IgA levels. IgM are the first antibodies to appear, usually 1 week after the infection. Toxoplasmic IgG appears after 2 weeks of infection and peaks at 3 months [3].

III. PRINCIPLE OF THE TEST

Scheme of the test procedure is a capture enzyme immunoassay. Microtiter strip wells precoated with the anti-human IgM. Serum IgM-antibodies are bound to the immobilized anti-human IgM. Unbound antibodies are removed by washing. The antigen of *T. gondii* conjugated to horseradish peroxidase (HRP) is bound to the prebounded specific IgM 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 anti-*T. gondii* IgM.

IV. CONTENT OF THE KIT abia Toxo IgM Capture

4.1 Contents of the reagent kit.

Table 1

LABEL	NATURE OF THE REAGENTS	PRESENTATION
Anti-human IgM Coated Strips	Polystyrene stripped 96-well plate (breakable wells) coated with the monoclonal antibodies to human immunoglobulin M. Store at 2-8 °C until expiration date.	1 plate
Conjugate	*T. gondii* antigen conjugated with HRP enzyme with addition of 1M Tris HCl buffer (pH 7.4-7.6) and bovine serum albumin (1.96%). Preserving agents: 0.10% ProClin 300, 0.10% phenol. Transparent or slightly opalescent yellow colored 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 specific antibodies to *T. gondii* with addition of 1M Tris HCl buffer (pH 7.4-7.6). Preserving agents: 0.10% sodium azide, 0.10% phenol. 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	Human serum, not containing anti-*T. gondii* IgM. 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
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, with addition of urea (10.57%) and bovine serum albumin (2.64%). Preserving agents: 0.10% ProClin 300, 0.01% 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

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.
- Never use the same container to distribute conjugate and color development solution.
- 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 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.
- Positive Control is prepared from the goat blood (inactivated), contains antibodies to Toxoplasma gondii.
- 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, Sample Diluent contain 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 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 0.2 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.
- Laboratory clock.
- Open type automated analyzer with 450 nm or with 450 and 620-680 nm filters (for automated procedure).

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. The samples after heat inactivation at 56 °C for 30 minutes cannot be analyzed.

Samples can be stored at 2-8 °C not more than for 8 days; they may be deep-frozen at -20°C. Samples that have been frozen and defrosted more than 1 time cannot be used.

Samples with expressed bacterial growing, hemolysis, hyperlipidemia must not be analyzed.

IX. PREPARATION OF THE REAGENTS

1. Ready to use reagents:

- **Anti-human IgM 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;**
- **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, 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 serum (plasma) samples. Carefully mix by pipetting. Violet-blue color should change to blue-green.
2	Add 100 µl of Positive and Negative Controls into the wells. 1 strip – Positive Control to 1 well and Negative Control to 2 wells; 2 strips and more – Positive Control to 1 well and Negative Control 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.
8	Remove fluid from wells; wash the plate **4 times** as described in step 5.
9	Add 100 µl of TMB-Substrate into all the wells.
10	Incubate at 18-24 °C for a **20 min** in a dark place.
11	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 1.8;
2. **Negative Control**: the absorbance value should not be more than 0.2.

Calculate cut-off value as:

Cut-Off = average OD value of Negative Control + A (A = 0.200),

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

Interpretation of Results

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

XII. PERFORMANCE CHARACTERISTICS

1. Interferences

Hemoglobin (up to 43.24 mg/ml), bilirubin (up to 0.3 mg/ml), lipids (up to 11.4 mg/ml), rheumatoid factor (up to 221 IU/ml) have no influence on the assay results.

2. Cross reactivity

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

3. Diagnostic sensitivity

Diagnostic sensitivity of abia Toxo IgM Capture with 114 anti-*T. gondii* IgM positive samples is 94.7% (95% CI: 89.0-97.6).

4. Diagnostic specificity

Diagnostic specificity of abia Toxo IgM Capture with 128 anti-*T. gondii* IgM negative samples is 100.0% (95% CI: 97.0-100.0).

5. Trueness. Agreement with certified reference measurement procedure

The abia Toxo IgM Capture was compared with the "Anti-Toxoplasma gondii ELISA (IgM)", (EUROIMMUN AG). 331 serum and plasma samples are tested.

	Anti-Toxoplasma gondii ELISA (IgM)			
abia Toxo IgM Capture		Positive	Negative	Indeterminate
	Positive	117	11	8
	Negative	15	160	20

The abia Toxo IgM Capture has not a borderline range, so indeterminate results were not included in the calculation. The agreement to comparative assays is 91.4% (95%CI: 87.7-94.1%).

6. Precision

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

Intra-assay (within run) precision

Data	Sample #1	Sample #2	Sample #3
Mean (S/Co)	5.4	4.4	2.6
S_r	0.1	0.0	0.1
CV (%)	1.9	0.9	2.2

Inter-assay (between-run) precision

Data	Sample #1	Sample #2	Sample #3
Mean (S/Co)	5.4	4.4	2.6
S_{rr}	0.11	0.14	0.06
CV (%)	2.0	3.1	2.4

XIII. LIMITS OF THE TEST

- A positive test result indicate that there has been contact with the pathogen at some undetermined time.
- 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.
- 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.
- For diagnosis, the clinical picture of the patient always needs to be taken into accont along with the serological findings.
- 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.

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XV. REFERENCES

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3. Villard O., Cimon B., L'Ollivier C., Fricker-Hidalgo H. et al. Serological diagnosis of *T. gondii* infection: recommendations from the French National Reference Center for toxoplasmosis. Diagn Microbiol Infect Dis. 2016 Jan; 84(1): 22-33.

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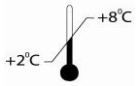
5. Abdel-Hameed D.M., Hassanein O. Evaluation of semi-quantitative PCR and IgG & IgM ELISA in diagnosis of toxoplasmosis in females with miscarriage. Egypt Soc Parasitol. 2004 Aug; 34(2): 559-70.

6. M36-A Vol.24, No.6: Clinical use and interpretation of serologic tests for Toxoplasma gondii, Approved Guideline, 2004.

XVI. EXPLANATION OF SYMBOLS

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

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
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