



TOXO

Detection system of *Toxoplasma gondii*'s DNA



Reg. MSP 21205

Valdense 3616. Montevideo, 11700. Uruguay.
Phone (598) 2 336 83 01.
Fax (598) 2 336 71 60.
info@atgen.com.uy
www.atgen.com.uy

 ATGen <small>DIAGNOSTIC SOLUTIONS</small>	Code: IDX-016	Ver: 1
---------------------------------------------------------------------------------------------------------------------------------------	----------------------	---------------

The purchase of this product does not provide a license to carry out patented applications.

Kit utility

The TOXO kit allows detecting the presence of *T.gondii*'s DNA in blood or amniotic fluid specimens, with an analytical sensitivity of 40 fg of DNA per PCR reaction.

Principle of the test

The test is based on the extraction of the specimen's DNA and subsequent PCR amplification of a specific 524 bp *T.gondii* DNA sequence, which is visualized in acrylamide gels.

The kit includes an amplification control in order to avoid false negative results.

The test can be completed in 8 hrs.

Introduction

The routine tests used for diagnosing toxoplasmosis are based on the indirect detection of the patient's immune response to the parasite *T. gondii*. Yet, there are certain situations in which the direct detection of *T.gondii* is advisable, in order to confirm diagnosis and adopt a therapeutic conduct.

This is the case of the confirmation of acute infection during pregnancy, congenital toxoplasmosis in newborns or children or the reactivation of a chronic infection in immune-depressed patients.

In these situations, PCR is an important diagnostic tool, which allows directly detecting the parasite's DNA in an easy, fast and specific way.

ATGen's test for the detection of *T.gondii*'s DNA in biological fluids is based on the PCR amplification of a specific 524 bp fragment of the parasite's DNA, which is present in high number of copies in the parasite genome.

Our results regarding sensitivity, demonstrate that it is possible to detect up to 1 tachyzoite per 500 µl of blood or amniotic fluid.

Our diagnostic system also includes an amplification control, as a means of controlling the formation of replicons with the primers used in the test. This control is based on the simultaneous amplification of the sample's DNA and a synthetic sequence which has a higher molecular weight than the *T.gondii*'s specific amplification band.

Validity of the test

The TOXO test allows detecting the presence of *T.gondii*'s DNA with an analytical sensitivity of 1 tachyzoite per 500 µl of blood or amniotic fluid.

NOTE: *T.gondii* load levels are low, both in blood and amniotic fluid. Thus it is recommended to process as much specimen volume as possible in order to avoid the apparition of false negative results (refer to specimen requirements).

Precautions

1. Only for in-vitro use.
2. All samples, reagents and controls should be considered as potentially infectious.
3. Do not use after the expiration date indicated on the package.



 ATGen <small>DIAGNOSTIC SOLUTIONS</small>	Code: IDX-016	Ver: 1
---------------------------------------------------------------------------------------------------------------------------------------	----------------------	---------------

Storage and Stability

The kit must be stored at -20°C in order to assure its optimal performance through the expiration date indicated on the package.

Specimen Requirements

Blood

2 - 5ml

Amniotic Fluid

2 - 10ml

Kit Presentation

The TOXO kit includes:

- 1 TOXO Reaction Mix tube containing the reaction mix for the amplification of a 524bp fragment which is present in *T. gondii*'s DNA.
- 1 *T. gondii* control DNA tube to be amplified with the TOXO Reaction Mix.
- 1 Control Reaction Mix tube, which includes the TOXO reaction mix primers and the vector containing the 663 bp amplification internal control.
- 1 TOXO Taq DNA polymerase tube.

The kit must be kept at -20 °C.

Our kits are available in two sizes: 20 and 50 reactions.

Necessary materials not provided with the test

- DNA-free PCR tubes
- Suitable Pipettes
- Filtered pipette tips
- Gloves.
- Thermocycler
- Cuvette for vertical electrophoresis
- Acrylamide, electrophoresis buffer and loading buffer
- Gel coloring system with silver nitrate.
- Vortex
- Bio-hazard disposable container

DNA Preparation

Extract DNA from the specimen (blood or amniotic fluid) using procedures that ensure high performance and purity. ATGen recommends performing the extraction by using ADN Facil kit or an extraction technique with phenol, chloroform and isoamyl alcohol.

The DNA is later re suspended in 10 µl of PCR water or TE (10mM Tris-HCl; 0.1mM EDTA; pH8) 10 minutes at 65°C or 24hrs at 4°C.

Protocol

Defrost the TOXO Reaction Mix and the Reaction Mix Control, shake vigorously using the vortex.

Perform all manipulations in cold room.

TOXO Reaction Mix

- 1. Add 17 µl of reaction mix per sample to be tested including the two controls.
- 2. Add to the reaction mix, 1 µl of DNA Taq polymerase per sample to be tested.

Reaction Mix Control

- 3. In a new PCR tube, add 17 µl of Control mix per sample to be tested.
- 4. Add 1µl of Taq DNA Polymerase per sample to be tested.

Amplification

- 5. Aliquot both mixes, dispensing 18 µl in properly labeled PCR tubes.
- 6. Add 2 µl of the DNA solution of each sample, to the PCR tubes containing each of the previously prepared reaction mixes (TOXO and Control).
- 7. In order to test the *T.gondii* 's DNA amplification, add 2 µL of TOXO Control DNA to a PCR tube containing the TOXO mix (**amplification positive control**).
- 8. Add 2 µL of water to a PCR tube containing the TOXO mix (**amplification negative control**).
- 9. Program the thermocycler as follows: 40 cycles at 94°C/0:30, 60°C/0:30, 72°C/0:30; an initial 5 minute denaturizing step at 94°C and a final 5 minute extension step at 72°C.
- 10. Place the tubes in the thermocycler when it reaches 94°C.

Obtention of test results

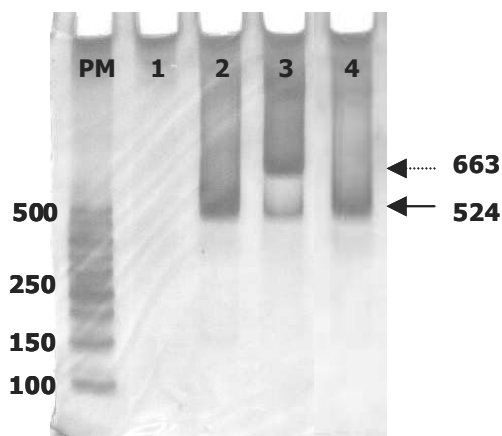
- Load 10-20 µl of each amplification product to a 6% acrylamide gel.
- Migrate until the xilenecianol blue dye has exited the gel.
- Dye with silver nitrate.

Optionally, the amplification can be tested loading 20 µl of each product and performing electrophoresis in 1% agarose gel dyed with ethidium bromure. However this method is less sensitive.

Interpretation of the test results

	<i>T.gondii</i>	Internal control (IC)
Amplified product	524 bp	663 bp

Example:



6% acrylamide gel developed with silver nitrate, showing a positive result obtained from amniotic fluid specimen.

Result:

Lane 1: Amplification negative control. Verify that there is no amplification.

Lane 2: Unknown sample, amplified with the TOXO mix. In this case, amplification is detected so the result must be informed as: presence of *T.gondii*'s DNA.

Lane 3: Unknown sample amplified with control mix: In this case amplification is detected in both bands: *T.gondii* (524 bp) and internal Control (663 bp).

Note: The internal control (IC) carries out the function of controlling the PCR reaction.

- If amplification is not observed with the TOXO mix and the IC amplifies correctly, the results are shown as: Absence of *T.gondii*'s DNA*.
- If amplification is not observed with the TOXO mix or with the Control mix, a flaw (presence of inhibitors in the sample or problems with the amplification) has occurred so the result is indeterminate.
- In cases as the example, when amplification is observed in lane 2, the result of lane 3 does not contribute with any additional information. In those cases when the *T.gondii* DNA is too abundant it could happen that only the 524 bp band can be seen with the Control Mix, yet this does not interfere with the result.

Lane 4: Amplification positive control. The specific *T.gondii* 524 bp band must be observed. This is an external control so it is not useful for the validation of the unknown sample result.

- When informing Absence of *T.gondii*'s DNA, the method's sensitivity limit must be specified (1 tachyzoite per 500 µl of blood or amniotic fluid).

Bibliography

Maniatis T., Fritsch E. & Sambrook J. Molecular cloning: A laboratory manual, 2nd Ed. 1989. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.