

ID Gene™ *Toxoplasma gondii* Duplex

Ref: IDTOXO-50 / IDTOXO-100

50 / 100 tests



Real-time PCR assay for **the qualitative detection of *Toxoplasma gondii***

Suitable samples: brain, placenta, heart muscle or swab supernatant (placental, vaginal and endocervical) from ruminants

In-vitro Use



December 2017:
Kit with a lyophilized Target Positive Control (TPC-TOXO) (previously PLS-TOXO)

General information

▪ Characteristics

ID Gene™ Toxoplasma gondii Duplex (IDTOXO) is a real-time PCR kit that amplifies a target sequence in *Toxoplasma gondii* genome, causative agent of Toxoplasmosis.

This kit is a qualitative duplex test. It simultaneously amplifies target DNA and an endogenous internal control.

The kit contains a target positive control (TPC-TOXO) which is to be extracted in the same manner as the samples to validate the extraction and amplification of the target.

This kit can be used to test brain, placenta, heart muscle homogenates or swab supernatant (placental, vaginal and endocervical) from ruminants.

▪ Kit composition and storage conditions

The IDTOXO kit contains the reagents shown below:

Reference	Component	Volume	Description
TPC-TOXO	Target Positive control	550 µl 1 vial	Inactivated <i>Toxoplasma gondii</i> , diluted in a negative brain matrix, freeze-dried and calibrated at between 10 and 100 times the detection limit of the method (MDL). Freeze-dried pellet to be reconstituted in 550 µl distilled or Nuclease-free water.
ARM-TOXO	Amplification Reaction Mix	400 µl 1 or 2 tubes (white cap)	Ready-to-use reaction mixture containing Taq polymerase and oligonucleotides for detection and amplification of <i>Toxoplasma gondii</i> and of the endogenous non-target positive control.

All components should be stored at ≤ -16°C. It is recommended to prepare aliquots (minimum 100 µl) in order to avoid multiple freeze/thaw cycles (> 3 not recommended).

▪ Material required but not provided in the kit

All material used should be of suitable quality for molecular biology.

Amplification Instrument:

- Real-time thermal cycler with channels capable of reading the following fluorophores: FAM, HEX or VIC and Cy5.

Examples of compatible thermal cyclers: CFX96™, Chromo4™ (Biorad), LC@480 I, LC@480 II, LC@96 Roche, AB@ 7500 and Rotor-Gene Q Qiagen. Please contact us regarding suitability with other thermal cyclers.

Consumables:

- Precision pipettes capable of delivering volumes of between 1 µl and 1000 µl
- Nuclease-free filtered tips
- 1.5 ml tubes
- 96-well PCR plates, strips or PCR micro-tubes (that have an optical quality compatible with the thermal cycler) and appropriate adapted adhesive film or caps
- Refrigerated rack

Reagents:

- Distilled or Nuclease-free water (recommended)

Remarks and precautions

The material used contains less than 0.1% hazardous or carcinogenic substances, thus MSDS sheets are not required. However, it is recommended to take appropriate precautions, as with any biochemical product, and to wear appropriate clothing.

Extraction controls

▪ Positive controls

The IDTOXO kit contains the following positive controls:

-Target Positive control (TPC-TOXO):

This control consists of inactivated *Toxoplasma gondii*, diluted in a negative brain matrix, freeze-dried and calibrated at between 10 and 100 times the detection limit of the method (MDL).

This control validates the efficiency of the extraction and amplification of the process.

This control is prepared and extracted in the same way as samples.

-Endogenous Non-Target Positive Control (NTPCen):

This control is constitutively present in the cells of the test sample. Its function is to validate (1) cell lysis and (2) amplification of a non-target gene. It also confirms the presence of cells, and gives an indication of the quality of the sample.

▪ **Negative controls**

It is recommended to include the following negative controls:

- Negative extraction control (NEC)

It is recommended to include a negative control for extraction (NEC) in each run.

This control should be prepared and extracted in the same way as samples, but should not contain any target DNA. The volume occupied by the sample is replaced by a non-reactive matrix or Nuclease-free water.

- Negative control for amplification (NAC)

This control contains 8 µl of reaction mix (ARM-TOXO) and 5 µl of Nuclease-free water. It is included in each analysis cycle to control for the presence of any aerosol contaminants.

▪ **Extraction of DNA**

The *Toxoplasma gondii* DNA must be extracted from the sample before being amplified by PCR.

For this, IDvet Genetics offers a range of extraction kits that meet the French standard AFNOR NF U47-600 requirements:

Description	Product name	Product code
Magnetic bead extraction system	ID Gene™ Mag Universal Extraction Kit	MAG192/MAG384
	ID Gene™ Mag Fast Extraction Kit	MAGFAST384
Column extraction system	ID Gene™ Spin Universal Extraction Kit	SPIN50/SPIN250

▪ **Extraction of control**

Volume of the control to extract is described in the table below:

Important:

- Volume indicated is valid regardless of the extraction system.
- Control must be extracted at the same time as the samples.

Control	Volume
TPC-TOXO	50 µl

Note: If the NEC is prepared with a negative sample matrix, refer to the extraction kit protocol for the matrix in question.

Amplification protocol

▪ **Preparation of the real-time PCR amplification reaction**

1. Prepare an experimental plan for the analysis of the samples and controls, being sure to distance the positive control (TPC-TOXO) from the other samples.
2. Thaw the IDTOXO kit, ideally at 5°C (± 3°C) in a refrigerated rack. Thaw at room temperature 21°C (± 5°C) only if the mix is to be used immediately after thawing.
3. Homogenise the contents of the **ARM-TOXO** tube by vortexing. Centrifuge down briefly.
4. Distribute **8 µl of ARM-TOXO** per well. Use PCR strips, or microplates adapted to the thermal cycler in use.
5. Add :
 - 5 µl of DNA extracted from each sample to be analyzed
 - 5 µl of TPC-TOXO
 - 5 µl of extracted NEC
 - 5 µl of Nuclease-free water (NAC)
6. Cover the plate or strips with appropriate adhesive film or caps.

▪ **Programming the amplification phase**

1. Program the thermal cycler detectors to read the following wavelengths for each well:

Target	Channel capable of reading	Quencher
<i>Toxoplasma gondii</i>	FAM	non fluorescent*
<i>NTPCen</i>	VIC/HEX	non fluorescent* (compatible VIC/HEX)

Note: For devices requiring an internal reference for optical calibration, the amplification mix ARM-TOXO contains ROX.

*Using a TAMRA™ quencher can improve the data analysis with some instruments.

2. Choose between the two 2 different amplification programs validated by IDvet Genetics:
 - Standard program (allows for PCR kits from different vendors to be used in a single run),
 - or
 - Rapid program

Step	Standard program	Rapid program	Number of cycles
(1) Polymerase activation	10 min at 95°C	2 min at 95°C	1
(2) DNA denaturation/elongation	15 sec at 95°C	10 sec at 95°C	40
	60 sec at 60°C	30 sec at 60°C	

Note: The fluorescence is read at the end of the elongation phase at 60°C.

3. Enter one or these programs in the thermal cycler and select a final volume of **13 µl per PCR**. If different volumes are combined in a single run, enter the largest volume on the plate.
4. Place the PCR plate, PCR strips or capillaries in the thermal cycler and start the program.

Validation and interpretation of results

Assay validation

The analysis of results is based on the Cq (Quantification cycle) value of each sample that is obtained by each detector. The Cq is also known as the Ct value (Threshold cycle).

The test is validated according to criteria outlined in the table below. **Results should not be interpreted if any of these criteria are not met.**

Control	Expected result	Acceptability criteria
TPC-TOXO	Detected in FAM and VIC/HEX	Presence of a characteristic curve Refer to the Cq value indicated in the quality control certificate
NTPCen	Detected in VIC/HEX in each sample	Presence of a characteristic curve
NEC	Nothing detected if water used	Complete absence of a characteristic curve
	Detected in VIC/HEX if negative sample used	Presence of a characteristic curve
NAC	No detection	Complete absence of a characteristic curve

Note: TPC-TOXO may be used to monitor variations in analytical sensitivity as it is calibrated at between 10 and 100 times the MDL.

Suggested interpretation of results

For each sample, results may be interpreted according to the following criteria:

Sample	TOXO signal	NTPCen signal	Interpretation
Individual	Detected	Detected or not detected	Animal detected as positive for <i>Toxoplasma gondii</i>
	Not detected	Detected	Animal not detected for <i>Toxoplasma gondii</i>
	Not detected	Not detected	A problem occurred during sample distribution or extraction process / PCR reaction was inhibited

Non-validated samples:

- If the NTPCen is not detected but the sample is detected positive for *Toxoplasma gondii*, consider the sample as positive.
- If the NTPCen is not detected:
 - A problem occurred during sample distribution or during the extraction process. In this case, the sample is to be extracted again.
 - Or the PCR reaction was inhibited. In this case, perform a new amplification run following the procedure below.

Procedure to follow if the PCR reaction was inhibited:

1. Dilute the extracted DNA 10 times in Nuclease-free water.
2. Repeat the amplification step on 5 µl of this dilution.
3. If the NTPCen is detected, interpret the sample according to table above.
4. If the NTPCen is not detected, re-extract the sample or consider it uninterpretable.

Documentation and support

For questions or technical support, please contact: support.genetics@id-vet.com

For additional information, visit www.id-vet.com