VetMAX™ T. gondii Kit

TaqMan® real-time PCR for detecting *Toxoplasma gondii*

Catalog Number TXP50

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Technology	Species	Nucleic acid isolated from matrices	Test type
Real-time PCR (DNA) – Duplex – Endogenous IPC	Bovine Small ruminants (goat, sheep)	Organs (placenta, brain, heart muscle)	Individual

⚠ **WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

⚠ **WARNING! POTENTIAL BIOHAZARD.** Read the biological hazard safety information at this product's page at **thermofisher.com.** Wear appropriate protective eyewear, clothing, and gloves.

Information about the product

Description of the product

The **Applied Biosystems™ VetMAX™ T. gondii Kit**, developed in partnership with the Deux Sèvres Departmental Veterinary Laboratory, is a molecular diagnostic tool that enables detection of *Toxoplasma gondii* by real-time PCR.

Each DNA sample obtained after extraction is analyzed in a single well: the same well is used to specifically detect the DNA of the parasite *Toxoplasma gondii* and an IPC (Internal Positive Control). A positive IPC reflects both the efficiency of extraction and the absence of inhibitor in the samples.

The kit may be used on parasite DNA extracted from **organs**: placenta, brain, and heart muscle.

Complete protocols for parasite DNA extraction from these matrices are available upon request from Technical Support.

Kit contents and storage

The **VetMAX™ T. gondii Kit** contains reagents for detection of both *Toxoplasma gondii* and an IPC. Upon receipt, the entire kit should be stored at **–30°C to –10°C**. After initial use of a component, store it according to the following recommendations:

Component	Description	Volume (50 reactions)	Storage	
			Upon receipt	After initial use
3 - Mix Toxo (Green tube)	Mix for TaqMan® PCR. Contains: • The detection system for the *Toxoplasma gondii* target, including a TaqMan® probe labeled **FAM™ - TAMRA™**. • The detection system for IPC, including a TaqMan® probe labeled **VIC™ - TAMRA™**. • Buffer and real-time PCR enzyme.	2 × 500 µL	–30°C to –10°C	2°C to 8°C
4a - EPC Toxo (Brown tube)	**External Positive Control:** Positive control for *Toxoplasma gondii*. It consists of **already extracted** nucleic acid to be amplified during real-time PCR.	90 µL	–30°C to –10°C	–30°C to –10°C

Extraction and amplification controls

The **VetMAX™ T. gondii Kit** contains one control used to validate the amplification of the parasite DNA:

4a - EPC Toxo: positive control for *Toxoplasma gondii*

Already extracted positive control to be amplified during real-time PCR.

A positive result within the specified C_t range validates the amplification of the *Toxoplasma gondii* target by real-time PCR.

Validation of nucleic acid extraction for each sample is done by detection of an **endogenous IPC** (Internal Positive Control), **present in each sample**.

A positive IPC result with a compliant C_t value in a sample validates the extraction of this sample, whether positive or negative for the target pathogen, enabling elimination of false negatives and verification of the inhibitor effect.

We recommend including two negative controls to confirm correct analysis:

NCS: negative extraction control

This control consists of components used in the extraction without addition of the sample (the sample volume can be replaced by the buffer used in the sample preparation or by DNase/RNase-free water), and undergoes the same treatment as the samples: nucleic acid extraction and real-time PCR.

A negative result for *Toxoplasma gondii* and the endogenous IPC confirms the absence of contamination during the extraction and the real-time PCR.

NC: negative amplification control

This is the amplification mix distributed to the plate during the preparation of the real-time PCR, with 5 µL of DNase/RNase-free water added to adjust the reaction to 25 µL.

A negative result for *Toxoplasma gondii* and the IPC confirms the absence of contamination during real-time PCR reaction preparation.

Materials required but not provided

Unless otherwise indicated, all materials are available through **thermofisher.com**.

- Adjustable micropipettes (range of 1 µL to 1000 µL) with DNase/RNase-free filtered tips
- DNase/RNase-free water
- 1X TE buffer
- 1X PBS buffer
- A real-time PCR thermal cycler capable of detecting the following fluorophores:
  - FAM™ (emission maximum: λ515 nm)
  - VIC™ (emission maximum: λ554 nm)
- Optical-quality consumables compatible with the thermal cycler used: PCR 96-well plates, PCR strips (8 or 12 wells), microtubes or capillaries; suitable plate covers or caps for capping

Analysis procedure

The real-time PCR reaction volume is 25 µL:

- **3 - Mix Toxo**: 20 µL per reaction
- **Extracted DNA:** 5 µL per reaction

Extraction of parasite DNA

DNA must be isolated from the samples prior to real-time PCR analysis.

NOTE: For information about extraction methods that are compatible with and validated for the VetMAX™ T. gondii Kit, please contact Technical Support.

Preparation of the real-time PCR

1. Create an analysis plan for distribution of the mixes and samples. Keep the positive control (EPC) away from the other samples if possible.
2. Thaw **3 - Mix Toxo** at **2°C to 8°C**, **on ice** or on a refrigerated rack.
3. Thoroughly mix **3 - Mix Toxo** by shaking gently, then centrifuge briefly.
4. Add **20 µL of 3 - Mix Toxo** to each PCR plate well, PCR strip or capillary used.
5. Add the sample and control DNA to each reaction mix according to the pre-defined analysis plan:

Type of analysis	Component	Sample volume
Sample for analysis	DNA extracted from the sample	5 µL
Positive amplification control	**4a - EPC Toxo**	5 µL
Negative lysis control (NCS)	Extracted NCS	5 µL
Negative amplification control (NC)	DNase/RNase-free water	5 µL

6. Cover the PCR plate, PCR strips or capillaries with an adhesive plate cover or suitable caps.

Amplification by real-time PCR

1. Create the following detectors on the thermal cycler:

	Reporter	Quencher
Toxo (*Toxoplasma gondii*)	FAM™	TAMRA™[1]
IPC Toxo	VIC™	TAMRA™[1]
Passive reference: ROX™[1]		

[1] The fluorophores TAMRA™ and ROX™ are required for real-time PCR analysis if the thermal cycler is capable of detecting them. For other thermal cyclers, absence of the ability to detect these fluorophores does not affect the real-time PCR analysis.

2. Assign the **TOXO** detector and the **IPC TOXO** detector to each sample well used in the analysis.
3. Create the following real-time PCR program for the analysis:

	Step repetitions	Temperature	Duration
Step 1	×1	50°C	2 minutes
Step 2	×1	95°C	10 minutes
Step 3	×45	95°C	15 seconds
		60°C[1]	1 minute

[1] Collection of fluorescence data during the 60°C – 1 minute stage.

4. Place the PCR plate, the PCR strips or the capillaries in the thermal cycler and run the real-time PCR.

Analysis of the results

Analysis of the raw data

Refer to the recommendations of the thermal cycler manufacturer for the analysis of the raw data.

1. Position the threshold limits separately for each target of the real-time PCR.
2. For each detector, interpret the results according to the sample C_t values obtained as recommended below.

Validation

The test is validated if the following criteria are met:

	TOXO detector	IPC TOXO detector	Validation
EPC Toxo	$C_t = C_{t\ QC}$ TOXO of **4a - EPC Toxo** ± 3C_t[1]	$C_t < 45$ or $C_t > 45$[2]	PCR validated
NCS	$C_t > 45$	$C_t > 45$	Extraction validated
NC	$C_t > 45$	$C_t > 45$	PCR reagents validated

[1] Refer to the values listed in section 2.1 "EPC" of the Certificate of Analysis of the lot used for the test.
[2] The IPC value in the EPC should not be used for test validation.

Interpretation of results

For each sample analyzed, the results should be interpreted as shown below:

TOXO detector	IPC TOXO detector	Interpretation
$C_t < 45$	$C_t < 45$ or $C_t > 45$	*Toxoplasma gondii* detected
$C_t > 45$	$C_t < 45$	*Toxoplasma gondii* not detected
$C_t > 45$	$C_t > 45$	Not validated[1]

[1] The sample will be returned as not validated due to the negative IPC.

Procedure for handling non-validated samples

1. Dilute the sample DNA at a 1:10 dilution in 1X TE buffer.
2. Perform a new PCR analysis on 5 µL of this dilution.
3. If the diluted DNA is positive for *Toxoplasma* or negative for *Toxoplasma* with a compliant IPC result, the obtained result is then validated.
4. If the diluted DNA is negative for *Toxoplasma* with a non-compliant IPC result, the obtained result is still not validated. In this case, repeat the nucleic acid extraction using a sample pre-diluted 1:10 in 1X PBS buffer before extraction.
5. If the result is still not validated, repeat the analysis on a new sample.

Documentation and support

Customer and technical support

Technical support: visit **thermofisher.com/askaquestion**

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Order and web support
- User guides, manuals, and protocols
- Certificates of Analysis
- Safety Data Sheets (SDSs; also known as MSDSs)

  NOTE: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **thermofisher.com/support**.

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The information in this guide is subject to change without notice.

Revision history of Pub. No. MAN0008900 (English)

Revision	Date	Description
C.0	24 August 2017	Updated to the current document template, with associated updates to the warranty, trademarks, and logos.
B.0	12 October 2015	Corrected the list of species compatible with the kit. Added revision history table.
A.0	5 May 2014	Life Technologies format document.