VetMAX™ Campylobacter spp. Kit

Real-time PCR TaqMan® for the detection of *Campylobacter* spp.

Catalog Number CAMPP50

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Technology	Species	Nucleic acid isolated from matrices	Test type
Real-time PCR (DNA) – Duplex – Exogenous IPC	Bovine Small ruminants (sheep, goat) Wild ruminants	Organs (placenta) Placental swab Fetus Fetal fluid Feces	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™ Campylobacter spp. Kit** is a molecular diagnostic tool enabling real-time PCR detection of *Campylobacter* spp.

Each DNA sample obtained after extraction is analyzed in a single well: the same well is used for specific detection of the bacterial DNA of *Campylobacter* spp. and an IPC (Internal Positive Control). A positive IPC reflects both the efficiency of extraction and the absence of inhibitor in the samples.

It can be used on bacterial DNA extracted from **placenta, placental swabs, fetal tissue and liquid, and feces.**

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

Kit contents and storage

The **VetMAX™ Campylobacter spp. Kit** contains reagents for the detection in duplex of *Campylobacter* spp. and an IPC. Upon receipt, the whole kit must 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 tests)	Storage	
			Upon receipt	After initial use
1 - Sequences CAMP (Green tube)	Sequence pool (primers and probes). Contains: • The detection system for the *Campylobacter* spp. target, including a TaqMan® probe labeled **FAM™ - TAMRA™**. • The detection system for IPC, including a TaqMan® probe labeled **VIC™ - TAMRA™**.	100 µL	–30°C to –10°C	–30°C to –10°C
2 - Master Mix CAMP (White tube)	Mix for TaqMan® real-time PCR. Contains the buffer and the real-time PCR enzyme.	625 µL	–30°C to –10°C	2°C to 8°C
4a - EPC CAMP (Brown tube)	**External Positive Control:** Positive control for *Campylobacter* spp. 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
5 - IPC CAMP (Yellow tube)	**Internal Positive Control:** Exogenous internal control **to be added to each sample and each control** in the lysis step of the extraction.	250 µL	–30°C to –10°C	–30°C to –10°C

NOTE: For small extraction series, it is recommended that the IPC CAMP be aliquoted when first used to avoid more than 3 cycles of freezing/thawing (in a minimum volume of 50 µL).

Extraction and amplification controls

The **VetMAX™ Campylobacter spp. Kit** contains two controls, enabling validation of the extraction and the amplification of the bacterial DNA:

4a - EPC CAMP: positive control for *Campylobacter* spp.

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

A positive result within the specified C_t range is used to validate the amplification of the *Campylobacter* spp. target by real-time PCR.

5 - IPC CAMP: internal extraction control

Positive control **to be added to each sample in the lysis step** of the nucleic acid extraction.

A positive IPC result with a value within the acceptable C_t range in a sample validates the extraction of this sample, whether positive or negative for the target pathogen: 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 reagents used in the extraction without addition of the sample (sample volume can be replaced by the buffer used in the sample preparation or by DNase/RNase-free water) that undergoes the same treatment as the samples: nucleic acid extraction (with IPC added), then real-time PCR.

A negative result for *Campylobacter* spp. enables the validation of the absence of contamination during the extraction and the real-time PCR.

NC: negative amplification control

This is the amplification mix deposited on 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 *Campylobacter* spp. and IPC enables validation of 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**.

- Precision 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™ (maximum emission: λ515 nm)
  - VIC™ (maximum emission: 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:

- **Mix CAMP**: 20 µL per reaction. To be reconstituted extemporaneously before real-time PCR.
- **Extracted DNA:** 5 µL per analysis.

Extraction of bacterial DNA

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

Add **5 µL** of **5 - IPC CAMP** to each sample to be extracted and the NCS in the lysis step of the nucleic acid extraction.

NOTE: To learn about compatible and validated extraction methods for the VetMAX™ Campylobacter spp. Kit, please contact Technical Support.

Reconstitution of the reaction mix

Reconstitute the **Mix CAMP** just before use, in a room dedicated to preparation of mixes:

1. On first use, thaw the tube of **2 - Master Mix CAMP** at **2°C to 8°C on ice** or on a refrigerated rack. Store and maintain at **2°C to 8°C** for further use.
2. Thaw the tube of **1 - Sequences CAMP** at room temperature. Return it to between **–30°C to –10°C** after use.

3. Reconstitute **Mix CAMP** at **2°C to 8°C on ice** or on a refrigerated rack, according to the following calculation tables:

Component	For 1 reaction	For N reactions[1]
1 - Sequences CAMP	2 µL	N × 2 µL
2 - Master Mix CAMP	12.5 µL	N × 12.5 µL
DNase/RNase-free water	5.5 µL	N × 5.5 µL
Total volume	20 µL	N × 20 µL

[1] It is recommended to allow for an additional reaction with respect to the total number of reactions to be carried out during the analysis (samples and controls). Never mix components from different lots of kits (see Certificate of Analysis).

4. After reconstitution, start the real-time PCR immediately. Keep **Mix CAMP** at **2°C to 8°C** on ice or on a refrigerated rack until used.

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. Homogenize the **Mix CAMP** tube by gentle agitation, then briefly centrifuge.
3. Add **20 µL of Mix CAMP** to each PCR plate well, PCR strip or capillary.
4. Add DNA from samples and controls to the reaction mix according to the following preset analysis plan:

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

5. 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
CAMP	FAM™	TAMRA™[1]
IPC CAMP	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 analysis by real-time PCR.

2. Assign the **CAMP** detector and the **IPC CAMP** detector to each sample well used in the analysis.
3. Set up 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:

	CAMP detector	IPC CAMP detector	Validation
EPC CAMP	$C_t = C_t$ QC CAMP of **4a - EPC CAMP** ± 3C_t[1]	$C_t < 45$ or $C_t > 45$[2]	PCR validated
NCS	$C_t > 45$	$C_t = C_t$ QC IPC of **5 - IPC CAMP** ± 3C_t[3]	Extraction validated
NC	$C_t > 45$	$C_t > 45$	PCR components 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.
[3] Refer to the values listed in section 2.2 "IPC" of the Certificate of Analysis of the lot used for the test.

Interpretation of results

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

CAMP detector	IPC CAMP detector	Interpretation
C_t < 45	C_t < 45 or C_t > 45	*Campylobacter* spp. detected
C_t > 45	C_t ⩽ C_t IPC of NCS + 3C_t[1]	*Campylobacter* spp. not detected
C_t > 45	C_t > C_t IPC of NCS + 3C_t[1]	Not validated[2]

[1] Refer to the IPC C_t value obtained for the NCS done during the same extraction series as the samples to be analyzed. The IPC C_t value obtained for this NCS must first be validated as described above.

[2] The sample will be returned as not validated due to the non-compliant IPC value.

Procedure for handling non-validated samples

1. Dilute the DNA of the non-validated sample 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 *Campylobacter* spp. or negative for *Campylobacter* spp. with a compliant IPC result, the obtained result is then validated.
4. If the diluted DNA is negative for *Campylobacter* spp. with a non-compliant IPC result, the obtained result is still not validated. In this case, repeat the nucleic acid extraction using the 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. MAN0008720 (English)

Revision	Date	Description
B.0	24 May 2017	Updated to the current document template, with associated updates to the warranty, trademarks, and logos.
A.0	7 April 2014	Baseline for revision history