VetMAX™ A. phagocytophilum Kit

Real-time PCR TaqMan® for the detection of *Anaplasma phagocytophilum*

Catalog Number ANAP50

Doc. Part No. 100020305 **Pub. No.** MAN0008658 **Rev.** B.0

Technology	Species	Nucleic acid isolated from matrices	Test type
Real-time PCR (DNA) – Duplex – Exogenous IPC	Bovine Sheep Wild ruminants	EDTA blood Spleen Ticks	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™ A. phagocytophilum Kit** is a molecular diagnostic tool for real-time PCR detection of the 3 biovars of *Anaplasma phagocytophilum* (*phagocytophilum*, *equi* and HGE).

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 *Anaplasma phagocytophilum* and an IPC (Internal Positive Control). A positive IPC reflects both the efficiency of extraction and the absence of inhibitor in the samples.

This kit can be used on bacterial DNA extracted from **blood** in EDTA tubes, the **spleen** and **ticks**.

Complete protocols of bacterial DNA extractions from these matrices are available on request from Technical Support.

Kit contents and storage

The **VetMAX™ A. phagocytophilum Kit** comes in a box containing different reagents for the detection of *Anaplasma phagocytophilum* in duplex 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 reactions)	Storage	
			Upon receipt	After initial use
3 - Mix ANAP (Green tube)	Mix for TaqMan® PCR. Contains: • The detection system for the *Anaplasma phagocytophilum* target, including a TaqMan® probe labeled **FAM™ – NFQ** (Non-Fluorescent Quencher). • 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 ANAP (Brown tube)	**External Positive Control:** Positive control for *Anaplasma phagocytophilum.* 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 ANAP (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 ANAP 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™ A. phagocytophilum Kit** contains two controls, enabling validation of the extraction and the amplification of the bacterial DNA:

4a - EPC ANAP: positive control for *Anaplasma phagocytophilum*

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

A positive result within the specified C_t range enables validation of the amplification of the *Anaplasma phagocytophilum* target by real-time PCR.

5 - IPC ANAP: 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 addition of the IPC) and real-time PCR.

A negative result for *Anaplasma phagocytophilum* enables 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 *Anaplasma phagocytophilum* 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:

- **3 - Mix ANAP**: 20 µL per analysis
- **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 ANAP** 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™ A. phagocytophilum 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 the **3 - Mix ANAP** tube at **2°C to 8°C, on ice** or on a refrigerated rack.
3. Homogenize the **3 - Mix ANAP** tube by gentle agitation, then centrifuge briefly.
4. Add **20 µL of 3 - Mix ANAP** to each PCR plate well, PCR strip or capillary used.
5. Add the 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 ANAP**	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
ANAP	FAM™	NFQ (Non-Fluorescent Quencher)
IPC ANAP	VIC™	TAMRA™[1]
Passive reference: ROX™[1]		

[1] The fluorophores TAMRA™ and ROX™ must be entered 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 compromise the analysis by real-time PCR.

2. Assign the **ANAP** detector and the **IPC ANAP** 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:

	ANAP detector	IPC ANAP detector	Validation
EPC ANAP	$C_t = C_{t\ QC}$ ANAP of **4a - EPC ANAP** ± 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 ANAP** ± 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:

ANAP detector	IPC ANAP detector	Interpretation
C_t < 45	C_t < 45 or C_t > 45	*Anaplasma phagocytophilum* detected
C_t > 45	$C_t \leq C_t$ IPC of NCS + 3C_t[1]	*Anaplasma phagocytophilum* 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 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 *Anaplasma phagocytophilum* or negative for *Anaplasma phagocytophilum* with a compliant IPC result, the obtained result is then validated.
4. If the diluted DNA is negative for *Anaplasma phagocytophilum* 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 at 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. MAN0008658 (English)

Revision	Date	Description
B.0	29 June 2017	Updated to the current document template, with associated updates to the warranty, trademarks, and logos.
A.0	11 March 2014	Baseline for revision history