



AmpliTest *MycoplasmaVet* (Real Time PCR)

Diagnostic kit for detection of DNA sequences
specific for *Mycoplasma arginini*, *bovigenitalium*, *canis*, *edwardii*, *cynos*, *felis*,
gateae, *maculosum*, *molare*, *opalescens* and *spumans*
using the **Real Time PCR** technique

<i>Cat. No.</i>	<i>Quantity</i>
BAC35-100	100 assays

The diagnostic kit is intended for professional use in specialised clinical, diagnostic and research laboratories. We strongly recommend that you read the user manual attached to the diagnostic kit before first use.

TRANSPORT

The **AmpliTest *MycoplasmaVet* (Real Time PCR)** kit is transported in dry ice. Immediately unpack the kit after delivery. Immediately inform the manufacturer if the tape securing the transport box or the seal of the commercial packaging are damaged or there is no dry ice left in the styrofoam transport box.

KIT CONTENTS

Component	Description	BAC35-100 100 assays	Tube lid colour
RM	Reaction Mixture	2 × 550 µL	Green
PC	Positive Control	1 × 300 µL	Red
NC	Negative Control	1 × 300 µL	Blue
IC	Internal Control	2 × 750 µL	Transparent
WATER	Water	1 × 1500 µL	White

STORAGE AND TRANSPORTATION CONDITIONS

- The kit components should be stored at -20°C.
- **AVOID THE EXPOSURE OF THE RM COMPONENT TO LIGHT.**
- Avoid repeated freezing/thawing cycles of the kit components (especially the RM component). The kit is stable after 10 repeated freezing/thawing cycles.
- The kit is stable under recommended storage conditions until the expiry date stated on the label.
- Do not use the kit components after the expiry date.

PRODUCT DESCRIPTION

Application

The **AmpliTest *MycoplasmaVet* (Real Time PCR)** kit is designed to detect DNA sequences specific for *Mycoplasma arginini*, *bovigenitalium*, *canis*, *edwardii*, *cynos*, *felis*, *gateae*, *maculosum*, *molare*, *opalescens* and *spumans* bacteria, which cause respiratory and genitourinary infections, as well as conjunctivitis and arthritis in animals. In rare cases, they can cause miscarriages, hemolytic anemia, and even meningitis. The presence of the pathogen-specific sequences are assayed in DNA samples extracted from animal tissues (cats, dogs, cows). The diagnostic kit is intended for *in vitro* diagnostics. The kit is a qualitative type: it is used to confirm or exclude the presence of bacterial DNA in the DNA preparations.

Method principle

The **AmpliTest *MycoplasmaVet* (Real Time PCR)** kit contains primers enabling amplification of the fragment of the *Mycoplasma arginini*, *bovigenitalium*, *canis*, *edwardii*, *cynos*, *felis*, *gateae*, *maculosum*, *molare*, *opalescens* and *spumans* genomes. Accumulating amplicons (the amplified fragments of bacterial DNA) are detected owing to the specific TaqMan[®] probe which is hydrolysed during DNA synthesis. After hydrolysis, the FAM fluorescent dye is released from the probe, which is detected by the optical system of the Real Time PCR device. Carefully designed, the probe and primers ensure high specificity of the reaction.

To increase the reliability of the results, the **AmpliTest *MycoplasmaVet* (Real Time PCR)** kit contains an internal control system that can be used to monitor the proper course of nucleic acid extraction and the Real Time PCR reaction. In addition to the probe and primers for detection of the pathogen-specific sequences, the **RM** component contains the probe and primers for amplifying and detecting the internal control. Internal control detection occurs due to the release of the HEX fluorescent dye from the probe. A positive result for internal control amplification confirms the proper course of nucleic acid extraction and the Real Time PCR reaction.

ADDITIONAL INSTRUMENTS AND MATERIALS

The **AmpliTest *MycoplasmaVet* (Real Time PCR)** kit allows a complete diagnostic procedure (detecting the presence of *Mycoplasma arginini*, *bovigenitalium*, *canis*, *edwardii*, *cynos*, *felis*, *gateae*, *maculosum*, *molare*, *opalescens* and *spumans* bacteria in the samples) **only** in conjunction with the kit for DNA extraction from the sample and the Real Time PCR device.

NOTE 1: The **AmpliTest *MycoplasmaVet* (Real Time PCR)** kit is compatible with Real Time PCR devices having the detection of HEX and FAM fluorescent dyes;
A full list of compatible Real Time PCR devices can be found on the manufacturer's website.

NOTE 2: The **AmpliTest *MycoplasmaVet* (Real Time PCR)** kit does not contain the ROX reference dye.
For devices requiring normalisation, the ROX dye should be added to the RM component at the appropriate concentration. The ROX dye is not included in the kit.

TECHNICAL SPECIFICATION

Quality control	Complies with ISO 13485: Medical devices - Quality management systems
Optimal DNA amount added to the reaction	To 400 ng

DNA EXTRACTION

For DNA extraction and purification the silica-based DNA extraction kits are recommended. These kits ensure good-quality of DNA samples. DNA samples should be stored at 2–8°C (short storage period), at -20°C or at a lower temperature (long storage period). The amount of the material required depends on DNA extraction method.

The internal control system included in the kit can be used to control the DNA extraction process. For this, the **IC** component should be added to the sample at the initial stage of DNA extraction. The volume of the **IC** component depends on the volume of the elution buffer used for DNA extraction. Add 5 µL of the **IC** component for every 50 µL of the elution buffer.

NOTE 3: Samples should be collected in sterile tubes. Prior to DNA extraction, samples must be stored for a period and under conditions that guarantee the stability of the genetic material of the pathogen.

NOTE 4: Other DNA extraction methods can cause contamination of DNA preparations with compounds that significantly reduce the efficiency of the PCR reaction. This results in lower sensitivity of the assay and, in extreme situations, in complete inhibition of DNA amplification.

REAL TIME PCR REACTION

1. Determine the number of DNA preparations to be analysed (**n**).
2. Thaw the kit components. After thawing, mix the tubes thoroughly and briefly spin them.

Store thawed components at 2–8°C or in ice.

AVOID THE EXPOSURE OF THE RM COMPONENT TO LIGHT.

3. Determine the amount of DNA preparation added to the reaction (**x** µL).

NOTE 5: Optimal amount of DNA added to the reaction should not exceed 400 ng. Large amounts of DNA added to the PCR reaction may inhibit DNA polymerase activity and may reduce assay sensitivity.

OPTION I: The internal control was added to the sample during the DNA extraction process.

NOTE 6. Proceed according to Option I, if the internal control system is used to control the proper course of nucleic acid extraction and the Real Time PCR reaction.

- 4.1 Mix carefully $(n + 3) \times 11$ µL of the **RM** component and $(n + 3) \times (9 - x)$ µL of water.
- 4.2 Add $(20 - x)$ µL of the mixture to **n + 2** reaction tubes.
- 4.3 Add **x** µL of the DNA preparations to **n** reaction tubes. Add **x** µL of the **NC** component (negative control) to one of the two remaining reaction tubes and **x** µL of the **PC** component (positive control) to the other one. Then go to Step 5.

OPTION II: The internal control was not added to the sample during the DNA extraction process.

NOTE 7: Proceed according to Option II, if the internal control system is used only to monitor the proper course of the Real Time PCR reaction.

- 4.1 Mix carefully $(n + 3) \times 11$ µL of the **RM** component and $(n + 3) \times (8.5 - x)$ µL of water.
- 4.2 Add $(19.5 - x)$ µL of the mixture to **n + 2** reaction tubes.
- 4.3 Add **x** µL of the DNA preparations and **0.5** µL of the **IC** component (internal control) to **n** reaction tubes.
- 4.4 Add $(x + 0.5)$ µL of the **NC** component (negative control) to one of the two remaining reaction tubes $(x + 0.5)$ µL of the **PC** component (positive control) to the other one. Then go to Step 5.
5. Close the reaction tubes and centrifuge them shortly.
6. Place the reaction tubes into the reaction device and run the amplification program according to the following amplification profile.

Step	Temperature	Time	Data Collection	Cycles
Initial denaturation	95°C	5 min		1
Denaturation	95°C	10 s		} 40
Amplification	58°C	25 s	FAM and HEX	

NOTE 8: It is recommended to prepare the Real Time PCR reaction in the laminar chamber to reduce the risk of reaction mixture contamination.

ANALYSIS EVALUATION

The table below shows the correct Real Time PCR result:

Type of Sample	FAM	HEX	Result
Positive Control	+	-	Valid
Negative Control	-	+	Valid
Assay 1	+	+	Positive
Assay 2	+	-	Positive
Assay 3	-	+	Negative

+ means the PCR product amplification recorded as a fluorescence increase with a Cq value <36

- means no PCR product amplification and no fluorescence increase

Results with Cq values greater than 36 should be treated as equivocal or negative.

The **positive** result confirms the presence of the sequences specific for *Mycoplasma arginini*, *bovigenitalium*, *canis*, *edwardii*, *cynos*, *felis*, *gateae*, *maculosum*, *molare*, *opalescens* and *spumans* bacteria in the sample. The **negative** result means the absence, or presence below the detection limit, of the sequences specific for *Mycoplasma arginini*, *bovigenitalium*, *canis*, *edwardii*, *cynos*, *felis*, *gateae*, *maculosum*, *molare*, *opalescens* and *spumans* bacteria in the sample.

ADDITIONAL REMARKS

- The TaqMan[®] probes have dark quenchers that do not generate additional fluorescent signals. For devices which require it (e.g. RotorGene 6000), select the **None** option as the quencher type.
- The Real Time PCR device should be calibrated (color compensation should be carried out) for fluorescent dyes.
- The total volume of the reaction mixture is 20 µL. Change of this volume may have a significant impact on some test parameters, including the achieved detection limit.

WARNINGS AND PRECAUTIONS

- Take special care during DNA extraction. All samples should be treated as **potentially infectious material**.
- DNA extraction and detection of the DNA sequences specific for *Mycoplasma arginini*, *bovigenitalium*, *canis*, *edwardii*, *cynos*, *felis*, *gateae*, *maculosum*, *molare*, *opalescens* and *spumans* bacteria should be done by properly trained personnel in a professional laboratory which is allowed to work with potentially infectious material. Special attention should be paid to prevent cross-contamination of the DNA preparations during DNA extraction.
- To minimise the false results, adhere to the following rules:
 - If possible, designate the separate stands for DNA extraction as well as preparation and performance of the Real Time PCR reaction;
 - Change protective clothing and gloves between the stages of DNA extraction and preparation of the Real Time PCR reaction;
 - Bench surfaces should be washed with DNA removing/destructive agents;
 - Use only sterile filter tips for automatic pipettes;

- The **PC** component should be added to the reaction mixture as the **last one**. Before adding it, if possible, close the tubes with the DNA preparations and the **NC** component;
- Do not eat, drink or smoke while using the kit;
- Wash your hands immediately after work with the kit;
- After contact of the skin or eyes with the kit components, gently wash them with water;
- It is recommended that you dispose of the kit after its expiry date or of any unused kit components as a laboratory waste.

TROUBLESHOOTING

Real Time PCR reaction

Problem			Possible cause	Suggestions
FAM	HEX			
Incorrect fluorescence reading for positive control	-	+	The NC component instead of the PC component was added to the reaction tube.	Repeat the Real Time PCR reaction for the positive control.
	-	-	No PC component added or the RM component is of poor quality.	Repeat the Real Time PCR reaction for the positive control. If you receive an incorrect result again, assume the poor quality of the RM component (improper storage, improper transport conditions or exceeding the expiry date).
Incorrect fluorescence reading for negative control	+	+	Contamination of the RM or NC component.	Repeat the Real Time PCR reaction for the negative control. If you receive an incorrect result again, assume the contamination of the RM or NC component.
	+	-	The PC component instead of the NC component was added to the reaction tube.	Repeat the Real Time PCR reaction for the negative control.
	-	-	No NC component added or the RM component is of poor quality.	Repeat the Real Time PCR reaction for the negative control. If you receive an incorrect result again, assume the poor quality of the RM component.
Incorrect fluorescence reading for the tested sample	-	-	No IC internal control was added at one of the assay steps.	Determine if the IC internal control was added at the intended stage of the assay. Repeat the Real Time PCR reaction with the internal control added to the reaction mixture.
			Low DNA extraction yield and/or Real Time PCR inhibitors present. Too high DNA concentration. Poor quality of the RM component.	Measure the concentration of the DNA preparation and, if necessary, repeat the reaction with less DNA added to the reaction. In the absence of amplification in the HEX channel for DNA preparations assayed and for the negative control, assume the poor quality of the RM component. In case of amplification in the HEX channel for the negative control and no amplification in the HEX channel for DNA preparations tested, assume contamination of the DNA preparation with PCR inhibitors or low efficiency of nucleic acid extraction.

Nucleic acid extraction

Problem	Possible cause	Suggestions
Significantly higher Cq values obtained in the HEX channel for the samples assayed in comparison with the Cq value obtained for the negative control ($\Delta Cq > 2$)	Low efficiency of DNA extraction from the sample assayed.	To determine the reasons for the low efficiency of DNA extraction, please contact the manufacturer of the nucleic acid extraction kit.

CUSTOMER SERVICE

- Any problems or irregularities that arise during the use of the diagnostic kit can be reported by phone or email.
- Orders for the **AmpliTest (Real Time PCR)** kit can be sent by email.

CONTACT

Support and customer care

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