VetMAX™ Ruminant Abortion Screening Kit

TaqMan® real-time PCR for multiplex detection of 8 major pathogens that cause abortions in ruminants (*Coxiella burnetii*, *Chlamydophila* spp., *Listeria monocytogenes*, *Salmonella* spp., *Campylobacter fetus*, *Leptospira* pathogenic serovars, *Anaplasma phagocytophila* and Bovine Herpes Virus type 4)

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Technology	Species	Nucleic acid isolated from matrices	Test type
Real-Time PCR (DNA) – 2 Duplex and 6 Simplex – Endogenous IPC	Ruminants	Placental, vaginal, and cervical swabs	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™ Ruminant Abortion Screening Kit** is a molecular diagnostic tool for detecting the principal agents that cause abortions in ruminants (*Coxiella burnetii*, *Chlamydophila* spp., *Listeria monocytogenes*, *Salmonella* spp., *Campylobacter fetus*, and *Leptospira* pathogènes, *Anaplasma phagocytophila*, and Bovine Herpes Virus type 4) by real-time PCR.

Each DNA sample obtained after extraction is analyzed in 8 single wells; each well is used to specifically detect the viral DNA of the pathogen in question 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 DNA extracted from **placental, vaginal, and cervical swabs**.

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

Kit contents and storage

The **VetMAX™ Ruminant Abortion Screening Kit** contains components that can be used for detecting these 8 pathogens and an IPC. Upon receipt, the whole 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 (25 reactions)	Storage	
			Upon receipt	After initial use
3 - Mix SAR Cox b. (Red tube)	2 mixes for TaqMan® PCR. Each contains: • The detection system for the pathogen target, including a TaqMan® probe labeled **FAM™ – NFQ** (Non-Fluorescent Quencher) or **FAM™ – TAMRA™**. • The detection system for IPC, including a TaqMan® probe labeled **VIC™ – TAMRA™**. • Buffer and real-time PCR enzyme	500 µL	–30°C to –10°C	–30°C to –10°C
3 - Mix SAR Chlam (Orange tube)		500 µL	–30°C to –10°C	–30°C to –10°C
3 - Mix SAR ANAP (Yellow tube)	6 mixes for TaqMan® PCR. Each contains: • The detection system for the pathogen target, including a TaqMan® probe labeled **FAM™ – NFQ** (Non-Fluorescent Quencher). • Buffer and real-time PCR enzyme	500 µL	–30°C to –10°C	–30°C to –10°C
3 - Mix SAR BHV4 (Green tube)		500 µL	–30°C to –10°C	–30°C to –10°C
3 - Mix SAR C. fetus (Blue tube)		500 µL	–30°C to –10°C	–30°C to –10°C
3 - Mix SAR Lepto (Purple tube)		500 µL	–30°C to –10°C	–30°C to –10°C
3 - Mix SAR Listeria (White tube)		500 µL	–30°C to –10°C	–30°C to –10°C
3 - Mix SAR Salmo (Black tube)		500 µL	–30°C to –10°C	–30°C to –10°C
4a - EPC SARP (Brown tube)	**External Positive Control:** Positive control for 8 pathogens. It consists of **already extracted** nucleic acid to be amplified during real-time PCR.	360 µL	–30°C to –10°C	–30°C to –10°C

Extraction and amplification controls

The **VetMAX™ Ruminant Abortion Screening Kit** contains one control used to validate the amplification of DNA.

4a - EPC SARP: pathogen target positive control

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 pathogen 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 value 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 components 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 and real-time PCR.

A negative result from the pathogen target and endogenous IPC confirms 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 the pathogen target and 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**.

- 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™ (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 SAR pathogen: 20 µL per analysis
- Extracted DNA: 5 µL per analysis and per mix

Extraction of DNA

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

NOTE: For information about extraction methods that are compatible with and validated for the VetMAX™ Ruminant Abortion Screening 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. For each mix used for the analysis:
   a. Thaw the tube of **3 - Mix SAR pathogen** at **2°C to 8°C on ice** or on a refrigerated rack.
   b. Mix the tube of **3 - Mix SAR pathogen** by shaking gently, then centrifuge briefly.
   c. Add **20 µL of 3 - Mix SAR pathogen** to each PCR plate well, PCR strip or capillary used.

3. 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 SARP**	5 µL
Negative extraction control (NCS)	Extracted NCS	5 µL
Negative amplification control (NC)	DNase/RNase-free water	5 µL

4. 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 9 detectors on the thermal cycler:

	Reporter	Quencher
COXB	FAM™	TAMRA™[1]
CHL, LIST, SALM, CF, ANAP, BHV4, LEPT	FAM™	NFQ (Non-Fluorescent Quencher)
IPC SARP	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 **corresponding** pathogen detector to each sample and, if the mix detects an IPC, the **IPC SAR** detector in the well used for 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:

	Pathogen detector	IPC SAR detector (for the IPC detecting mix)	Validation
EPC SARP	$C_t = C_{t\ QC}$ SAR of **4a - EPC SARP** ±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 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.

Interpretation of results

For each sample analyzed, the results should be interpreted as shown below: For mixes that do not detect the IPC, refer to the IPC C_t value obtained with the IPC detecting mixes.

Pathogen detector	IPC SARP detector	Interpretation
$C_t < 45$	$C_t < 45$ or $C_t > 45$	Pathogen target detected
$C_t > 45$	$C_t < 45$	Pathogen target 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 or negative for the pathogen target with a compliant IPC result, the obtained result is then validated.
4. If the diluted DNA is negative for the pathogen target 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**

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- 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.

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Laboratoire Service International (LSI) | 6 Allée des Écureuils | Parc Tertiaire du Bois-Dieu | 69380 Lissieu, France

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Revision	Date	Description
B.0	22 July 2019	Updated to the current document template, with associated updates to the warranty, trademarks, and logos.
A.0	24 March 2014	Baseline for revision history

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