VetMAX™ Xeno™ Internal Positive Controls and Assays for PCR and RT-PCR

USER GUIDE

Catalog Numbers A29761, A29762, A29763, A29764, A29765, A29766, A29767, and A29768

Publication Number MAN0014500

Revision D.0

For Laboratory Use Including Veterinary and Environmental Uses.

ThermoFisher SCIENTIFIC

Life Technologies Corporation | 2130 Woodward Street | Austin, Texas 78744 USA
For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: MAN0014500 D.0 (English)

Revision	Date	Description
D.0	12 December 2023	The regulatory statement was updated.
C.0	14 April 2016	Corrections to Troubleshooting were made (see Appendix A, "Troubleshooting").
B.0	1 January 2016	The regulatory statement was updated.
A.0	1 October 2015	New document created for VetMAX™ Xeno™ Internal Positive Controls and Assays for PCR and RT-PCR.

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

Contents

Product information

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Product description

Applied Biosystems™ VetMAX™ Xeno™ Internal Positive Controls (IPC) and Assays provide a complete solution to qualify the effectiveness of sample preparation and real-time PCR and RT-PCR workflows.

- Add VetMAX™ Xeno™ IPC to the lysis solution used for nucleic acid isolation, to serve as a positive control for recovery of nucleic acid.
- Additionally, use diluted VetMAX™ Xeno™ IPC as a positive control for PCR and RT-PCR.
- Multiplex VetMAX™ Xeno™ IPC Assay with the assay of interest, to detect Xeno™ IPC in test samples, to enable monitoring of nucleic acid recovery and PCR inhibition.

VetMAX™ Xeno™ IPC and VetMAX™ Xeno™ IPC Assays enable the user to distinguish true target negatives from samples affected by PCR inhibition.

VetMAX™ Xeno™ IPC is available in DNA and RNA formulations. VetMAX™ Xeno™ IPC Assays are available with VIC™ or LIZ™ reporter dyes, for flexibility in multiplex reactions. The assay formulations are optimized for use with challenging animal samples to detect RNA or DNA pathogens.

IPC and Assay type	Rquired item	Recommended workflow
Applied Biosystems™ VetMAX™ Reagents that do not include Xeno™ IPC (RNA or DNA), such as: • VetMAX™ NA and EU PRRSV Reagents (Cat. no. 4468465) • VetMAX™ *M. hyopneumoniae* Reagents (Cat. no. 4415218)	VetMAX™ Xeno™ IPC (RNA or DNA)	Workflow A (page page 6)
Applied Biosystems™ VetMAX™ Plus reagents that do not include Xeno™ Assay: • Master mix with Xeno™ DNA control: VetMAX™-Plus qPCR Master Mix (Cat. no. 4415327) • Master mixes with Xeno™ RNA control: – VetMAX™-Plus Multiplex One-Step RT-PCR Kit (Cat. no. 4415330) – VetMAX™-Plus One-Step RT-PCR Kit (Cat. no. 4415328)	VetMAX™ Xeno™ IPC Assay (VIC™ or LIZ™ dyes)	Workflow B (page page 7)

(continued)

IPC and Assay type	Rquired item	Recommended workflow
• Master mix reagents and assays that do not include Xeno™ IPC (RNA or DNA) and Xeno™ Assay: – Applied Biosystems™ PCR Master Mix: Path-ID™ qPCR Master Mix (Cat. no. 4388643) – Applied Biosystems™ RT-PCR Master Mixes, such as: – AgPath-ID™ One-Step RT-PCR Reagents (Cat. no. AM1005) – Path-ID™ Multiplex One-Step Kit (Cat. no. 4442136) • Assays that do not include Xeno™ Assay	VetMAX™ Xeno™ IPC (RNA or DNA) and VetMAX™ Xeno™ IPC Assay (VIC™ or LIZ™ dyes)	Workflow C (page page 8)

Kit contents and storage

Table 1 VetMAX™ Xeno™ Internal Positive Control RNA

Component	Cat. no. A29763 (100 rxns)	Cat. no. A29761 (500 rxns)	Storage
Xeno™ RNA Control (10,000 copies/µL)	250 µL	1250 µL	–25°C to –15°C
Nucleic Acid Dilution Solution	250 µL	1250 µL	

Table 2 VetMAX™ Xeno™ Internal Positive Control DNA

Component	Cat. no. A29764 (100 rxns)	Cat. no. A29762 (500 rxns)	Storage
Xeno™ DNA Control (10,000 copies/µL)	250 µL	1250 µL	–25°C to –15°C
Nucleic Acid Dilution Solution	250 µL	1250 µL	

Table 3 VetMAX™ Xeno™ Internal Positive Control – VIC™ Assay

Component	Cat. no. A29765 (100 rxns)	Cat. no. A29767 (500 rxns)	Storage
Xeno™ VIC™ Primer Probe Mix	110 µL	550 µL	–25°C to –15°C

Table 4 VetMAX™ Xeno™ Internal Positive Control – LIZ™ Assay

Component	Cat. no. A29766 (100 rxns)	Cat. no. A29768 (500 rxns)	Storage
Xeno™ LIZ™ Primer Probe Mix	110 µL	550 µL	–25°C to –15°C

Methods

Workflow A (add Xeno™ IPC to samples)

Follow this procedure when:

- The PCR/RT-PCR reagent mix contains Xeno™ Assay
- The samples do not contain Xeno™ IPC (DNA or RNA)

Lyse the samples

1. Add 2 µL (20,000 copies) per reaction of Xeno™ IPC (DNA or RNA depending on the nucleic acid isolated) to the lysis solution.
2. Perform the nucleic acid isolation according to the standard procedure.

Perform the PCR or RT-PCR reaction

1. Program the real-time PCR instrument according to the manufacturer's recommendation.
2. Dilute the Xeno™ IPC (DNA or RNA depending on the nucleic acid isolated) 10-fold (1,000 copies per µL) to use as a PCR control.
3. Assemble the PCR or RT-PCR reaction mix according to the master mix used, reaction size, and number of reactions.
4. Distribute the PCR or RT-PCR reaction mix to the wells of a PCR plate or to PCR tubes.
5. Add purified nucleic acid and controls according to the following table.

Reaction type	Component
Test sample	Purified nucleic acid
Positive control[1]	Diluted Xeno™ IPC
Negative control[1]	Nuclease-Free Water

[1] Use the same volume of control as of test sample.

6. Load the plate in the instrument and run the thermal cycling program.

Workflow B (add Xeno™ Assay to PCR/RT-PCR mix)

Follow this procedure when:

- The PCR/RT-PCR reagent mix does not contain Xeno™ Assay
- The samples contain Xeno™ IPC

Note: We recommend that you perform appropriate testing to evaluate the performance of your target assay in presence of the Xeno™ Assay (i.e. the performance of your target remains the same after addition of the Xeno™ Assay to the master mix).

Perform the PCR or RT-PCR reaction

1. Program the real-time PCR instrument according to the manufacturer's recommendation.

   IMPORTANT! Make sure to set up the reporter corresponding to the Xeno™ Assay used (VIC™ or LIZ™ dye).

2. Dilute the Xeno™ IPC (DNA or RNA depending on the nucleic acid isolated) 10-fold (1,000 copies per µL) to use as a PCR control.

3. Assemble the PCR or RT-PCR reaction mix according to the master mix used, reaction size, and number of reactions.

4. Add 1 µL of Xeno™ Assay per 25 µL of PCR or RT-PCR mix.

5. Distribute the PCR or RT-PCR reaction mix to the wells of a PCR plate or to PCR tubes.

6. Add purified nucleic acid and controls according to the following table.

Reaction type	Component
Test sample	Purified nucleic acid
Positive control[1]	Diluted Xeno™ IPC
Negative control[1]	Nuclease-Free Water

[1] Use the same volume of control as of test sample.

7. Load the plate in the instrument and run the thermal cycling program.

Workflow C (add Xeno™ IPC to samples and Xeno™ Assay to PCR/RT-PCR mix)

Follow this procedure when:

- The PCR/RT-PCR reagent mix does not contain Xeno™ Assay
- The samples do not contain Xeno™ IPC

Note: We recommend that you perform appropriate testing to evaluate the performance of your target assay in presence of the Xeno™ Assay (i.e. the performance of your target remains the same after addition of the Xeno™ Assay to the master mix).

Lyse the samples

1. Add 2 µL (20,000 copies) per reaction of Xeno™ IPC (DNA or RNA depending on the nucleic acid isolated) to the lysis solution.
2. Perform the nucleic acid isolation according to the standard procedure.

Perform the PCR or RT-PCR reaction

1. Program the real-time PCR instrument according to the manufacturer's recommendation.

   IMPORTANT! Make sure to set up the reporter corresponding to the Xeno™ Assay used (VIC™ or LIZ™ dye).

2. Dilute the Xeno™ IPC (DNA or RNA depending on the nucleic acid isolated) 10-fold (1,000 copies per µL) to use as a PCR control.
3. Assemble the PCR or RT-PCR reaction mix according to the master mix used, reaction size, and number of reactions.
4. Add 1 µL of Xeno™ Assay per 25 µL of PCR or RT-PCR mix.
5. Distribute the PCR or RT-PCR reaction mix to the wells of a PCR plate or to PCR tubes.
6. Add purified nucleic acid and controls according to the following table.

Reaction type	Component
Test sample	Purified nucleic acid
Positive control[1]	Diluted Xeno™ IPC
Negative control[1]	Nuclease-Free Water

[1] Use the same volume of control as of test sample.

7. Load the plate in the instrument and run the thermal cycling program.

Analyze results

The general process for analyzing results from real-time PCR and RT-PCR experiments is:

1. View the amplification plots for all reactions to make sure they appear normal.
2. Set the baseline and threshold values.
3. Use the relative standard curve or the comparative C_T method to analyze your data.

The details of data analysis depend on the real-time PCR instrument that you use; refer to the appropriate user guide for instructions on how to analyze your data.

A

Troubleshooting

Observation	Possible cause	Recommended action
Test samples **Details:** Xeno™ IPC—no or low signal Target—high signal	The Xeno™ Assay components are at limiting concentrations in the PCR. High levels of target in a sample can reduce amplification of Xeno™ IPC.	No action required. No or low signal from Xeno™ IPC is expected in a reaction that has a strong signal for the target.
Test samples **Details:** Xeno™ IPC—no or low signal Target—no signal or inconclusive-range signal	Poor nucleic acid recovery.	Check the C_T values of Xeno™ IPC in the negative extraction control. A high C_T value for Xeno™ IPC indicates that the nucleic acid recovery was poor. Repeat the purification of the original sample.
	The RNA samples contain inhibitors of RT-PCR.	Dilute purified nucleic acid two- and four-fold and check if the signal strength increases due to the dilution of inhibitors.

B

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit **thermofisher.com/support**.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
  www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
  www.who.int/publications/i/item/9789240011311

C Documentation and support

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - 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 at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.