HoopBio

# IFU EasyqPCR
# Enterococcus faecalis

CE

Producto fabricado por **Proquinorte S.A.** Edif 208, 48170 Zamudio, Vizcaya.

## Contents

# Chapter 1

## Product information

Refer to the QuantStudio™ 5 Real-Time PCR Instrument installation, Use, and Maintenance Guide (Pub No. MAN0017162) for detailed instructions on preparing and running Real-Time PCR (RT-PCR) experiments.

**Note:** For safety and biohazard guidelines, refer to the "Safety" section in the QuantStudio™ 5 Real-Time PCR Instrument Installation, Use, and Maintenance Guide (Pub No. MAN0017162).

## General information

*Enterococcus faecalis* is a Gram-positive bacteria in the Phylum *Firmicutes*. *Enterococcus faecalis* is associated with the gastrointestinal tract microbiota and hospital-acquired infections.

The EasyqPCR Enterococcus faecalis (This assay corresponds to CADT ID AIGJRP0) of Enterococcus faecalis. Real-Time PCR (RT-PCR) is a precise and sensitive technique for quality presence/absence of pathogen microorganisms.

## Intended use

This assay corresponds to CADT ID AIGJRP0. This assay detects different species/strains of *Enterococcus faecalis*.

## Materials supplied

| ASSAY | REACTIONS | REFERENCE |
|---|---|---|
| EasyqPCR Enterococcus faecalis | 50 | 07263854 |

## Required materials not supplied

| Real-Time PCR System* | ThermoFisher Scientific |
|---|---|
| QuantStudio™ 5 Real-Time PCR System, 96-well, 0.2 mL | A28569 |
| **Equipment** | **Proquinorte** |
| Centrifugue, bench top | 07411728 |
| Vortex mixer | 08085157 |
| SmartBlock 0.5mL | 07411691 |
| **Consumables** | **Proquinorte** |
| MagMAX CORE Kit | 07237252 / 07237254 |
| PBS (10X) pH 7.4 | 07238845 |
| P20 or P200 Pipettes | 07367058 / 07367052 |
| P20 or P200 Pipette tips | 00000149 / 00000151 |
| Microcentrifugue tubes / Microcentrifugue tube rack | 07368219 / 07440468 |
| 96-well plates MicroAmp™ | 07241784 |

## Chapter 2

### Prepare the RT-PCR reaction mix

VetMAX™ Xeno™ Internal Positive Control DNA serves as an internal positive control for the DNA purification process and helps monitor for the presence of PCR inhibitors in animal health molecular detection workflows.

EasyqPCR Enterococcus faecalis is positive in the FAM channel, VetMAX™ Xeno™ Internal Positive Control is a primer-probe mix that detects the Xeno internal positive control, positive in the VIC channel.

### Isolation Nucleic acid

1. Isolate DNA from each whole blood sample of interest with MagMAX CORE kit. Add 2 µL (20,000 copies) per reaction of VetMAX™ Xeno™ Internal Positive Control DNA to the lysis solution.

2. Perform the nucleic acid isolation according to the standard procedure.

### Dilution Xeno RNA Control (Positive control, C+)

1. Vortex the Nucleic Acid Solution tube (inside ref A29762) and add 90 µL to an Eppendorf tube.

2. Vortex the Xeno™ DNA Control tube (inside A29762) and add 10 µL to the Eppendorf tube containing 90 µL of Nucleic Acid Dilution Solution (make sure there are no droplets on the lid before transferring the volume).

3. This prepares the Xeno DNA Control for use as a Positive Control (C+). 100 µL is sufficient for 8 reactions.

### Prepare the Real Time PCR Mix

1. Mix the VetMAX™ Fast Multiplex Master Mix thoroughly. Ensure there are no droplets on the lid.

2. Mix the Enterococcus faecalis assay thoroughly. Ensure there are no droplets on the lid.

3. Mix the VetMAX™ Xeno™ Internal Positive Control VIC™ Assay thoroughly. Ensure there are no droplets on the lid.

4. In an Eppendorf tube, add the volumes listed below for the required number of reactions (take into account the Extraction Negative Control, NEC) the No Template Control (NTC), the Positive Control (C+) (diluted Xeno DNA, and the excess)

| Reagents | Volume per reaction (with 10% overage)1 |
|---|---|
| VetMAX™ Fast Multiplex Master Mix (2X) | 12,5 µL |
| Enterococcus faecalis assay (20X) | 2,5 µL |
| VetMAX™ Xeno™ Internal Positive Control VIC™ assay (25X) | 1 µL |
| Volume | 16 µL |

**Prepare the Real Time PCR Plate**

1. Dispense 16 µL of the prepared mix into the wells of a PCR Plate
2. Then add 8 µL of sample*
   * 8 µL of NEC,NTC and C+ are added to the corresponding wells
3. Final reaction volume: 25 µL (16 µL PCR Mix + 8 µL sample/NEC/NTC/C+ and fill up to 25 µL with nuclease-free water)

| Reagents | Volume per reaction (with 10% overage)1 |
|---|---|
| VetMAX™ Fast Multiplex Master Mix (2X) | 12,5 µL |
| Enterococcus faecalis assay (20X) | 2,5 µL |
| VetMAX™ Xeno™ Internal Positive Control VIC™ assay (25X) | 1 µL |
| Sample/NEC/NTC/C+ | 8 µL |
| Nuclease-free water | Up to 25 µL |
| Volume | 16 µL |

Note: Adjust the volume if you use 384–well plates or 96–well 0.1-mL plates.

**IMPORTANT!** For optimal results, prepare the reaction plate on ice.

[1] After calculating the number of reactions required, prepare RT-PCR mix for the appropriate number of reactions and scale those components by 10% for overage. Dilute assay accordingly to avoid pipetting less than 1 µL volumes.

# Enterococcus faecalis

**Chapter 3.**

## Set up and run the experiment

1. Seal the plate with a MicroAmp ™ Optical Adhesive Film, then vortex briefly to mix the contents.
2. Centrifuge the plate briefly to collect the contents at the bottom of the wells.

   **IMPORTANT!** Vortexing and centrifuging are required for proper mixing of the reaction components.

   **IMPORTANT!** Run the plate within 2 hours of preparation, or store the plate at 2–8°C for up to24 hours.
3. Program the real-time PCR instrument according to the manufacturer's recommendation.

   **Note:** The instrument must be configured with the block appropriate for the plate type.

   **IMPORTANT!** The cycling mode depends on the master mix that is used in the reaction. The cycling mode does not depend on the plate format.
4. Set up the thermal protocol (Fast cycling mode)

| Step | Temperature | Time | Cycles |
|---|---|---|---|
| Activation | 95°C | 10 minutes | 1 |
| Denaturation | 95°C | 3 seconds | 40 |
| Anneal/Extension | 60°C | 30 seconds | |

5. Set up channels for detection:
   Enterococcus faecalis: FAM
   VetMAX™ Xeno™ Internal Positive Control: VIC
   Passive Dye: ROX
6. Load the plate into the real-time PCR instrument. Start the run

**Analyze the results**

For more information about analyzing the results, see the appropriate resources that are listed in "Documentation and support".

Use the absolute or relative quantification (ΔΔCt) methods (without target normalization) to analyze results.

The general guidelines for analysis include:
• View the amplification plot; then, if needed:
– Adjust the baseline and threshold values.
– Remove outliers from the analysis.
• In the well table or results table, view the Ct values for each well and for each replicate group, if applicable.

## Documentation and support

### Related Documentation

| Document | Publication number | Description |
|---|---|---|
| QuantStudio™ 5 Real-Time PCR Instrument Installation, maintenance and administration | MAN0017162 | Detailed instructions for using QuantStudio™ 5 Real-Time PCR to installation, use and maintenance |
| Real-Time PCR Analysis Software | MAN0009819 | Real-Time PCR Analysis Software is designed to analyze and identification of nucleic acids |

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  - –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

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