



IFU EasyqPCR Enterococcus faecium



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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 faecium is a Gram-positive bacteria in the Phylum *Firmicutes*. *Enterococcus faecium* is associated with the gastrointestinal tract microbiota, but can also become pathogenic due to antibiotic resistance, in particular Vancomycin-resistance.

The EasyqPCR *Enterococcus faecium* (This assay corresponds to CADT ID APPRJ2H) of *Enterococcus faecium*. 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 APPRJ2H. This assay detects different species/strains of *Enterococcus faecium*.

Materials supplied

ASSAY	REACTIONS	REFERENCE
EasyqPCR <i>Enterococcus faecium</i>	50	07263855

Required materials not supplied

Real-Time PCR System*	ThermoFisher Scientific
QuantStudio™ 5 Real-Time PCR System, 96-well, 0.2 mL	A28569
Equipment	Proquinorte
Centrifuge, 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
Microcentrifuge tubes / Microcentrifuge 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 faecium 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 Enterobacteriaceae 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 faecium 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 faecium 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.

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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 to 24 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 faecium: 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 ($\Delta\Delta Ct$) 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

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

