BioGX
Molecular Made Easy

SRM11 - Enterococcus faecium, Enterococcus faecalis Open System PCR Reagents

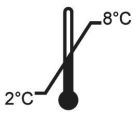
REF **450-082-LMP**

Σ 64 Reactions

Product Insert

For Research Use Only: Not intended for In Vitro Diagnostic Use

For use with ABI QuantStudio™ 5, ABI 7500 Fast Dx, Bio-Rad CFX96 Touch™, Bio-Rad CFX384 Touch™, BioGX pixl.16 real-time PCR platform



RUO

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For Research Use Only

Research use only reagents are not intended for human or animal diagnostic use. It is the responsibility of the end user to determine the performance of the reagents in an appropriately designed validation study for their intended use.

The Enterococcus faecium, Enterococcus faecalis real-time PCR-based detection reagent is manufactured and packaged as an open system reagent (OSR) for use with open system platforms and has to be validated by the user. Examples of open system platforms are the Applied Biosystems QuantStudio™ 5 (Design & Analysis software version 1.5.1 or later), Applied Biosystems 7500 Fast Dx (SDS software version 1.4 or later), Bio-Rad CFX96 Touch™, CFX384 Touch™ (Maestro software version 1.1 or later) or BioGX pixl.16 (For Android® based software version 1.6.9 or later) real-time PCR platforms.

PLEASE READ ENTIRE PACKAGE INSERT BEFORE PROCEEDING TO USE THE OSR.

PRODUCT OVERVIEW

The BioGX Sample-Ready™ OSR has been formulated in lyophilized format for the multiplex real-time PCR-based detection of DNA from Enterococcus faecium (16S rRNA gene[1]), Enterococcus faecalis (trmD gene[2]), and RNaseP which serves as an endogenous extraction control. The following format for the lyophilized Sample-Ready OSR kit is available:

1. ABI QuantStudio™ 5, ABI 7500 Fast Dx, Bio-Rad CFX96 Touch™, Bio-Rad CFX384 Touch™ and BioGX pixl.16 Platforms
   REF 450-082-LMP

Note:

OSR for ABI, Bio-Rad and BioGX pixl.16 Platforms (450-082-LMP) contains all PCR primers, probes, enzymes, dNTPs, $MgCl_2$, buffers, and other components required for the PCR reaction. No exogenous addition of SPC is needed.

PACKAGE CONTENTS

BioGX REF: 450-082-LMP
Platform(s): ABI QuantStudio™ 5, ABI 7500 Fast Dx, Bio-Rad CFX96 Touch™, Bio-Rad CFX384 Touch™ and BioGX pixl.16

Each 64-reaction package consists of one pouch:

The pouch contains 2 x 8-tube strips. Each tube contains BioGX lyophilized Sample-Ready™ OSR sufficient for 4 x 15 µL PCR reactions.

Note: 8-tube strips containing reagents **ARE NOT** compatible with ABI QuantStudio™ 5, ABI 7500 Fast Dx, Bio-Rad CFX96 Touch™, Bio-Rad CFX384 Touch™ and BioGX pixl.16 instruments.

EQUIPMENT AND MATERIALS REQUIRED BUT NOT PROVIDED

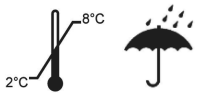
- Lyophilized Positive Control Template DNA Beads (10^5 copies/bead)
  - BioGX Enterococcus faecium (Part number 720-0122)
  - BioGX Enterococcus faecalis (Part number 720-0236)
- Lyophilized Positive Control Template DNA Beads (10^5 copies/bead) for ABI and Bio-Rad platforms
  - BioGX RNaseP (DNA) (Part number 720-0009)
- BioGX Molecular Grade Water or equivalent
  - BioGX Rehydration Water (Part number: 800-0035-12)
- Vortex Genie 2 Vortexer (VWR catalog no. 58815-234) or equivalent
- Disposable nitrile gloves
- Applied Biosystems QuantStudio 5 (0.2 mL) consumables.
  - Thermo Fisher optical 8-tube strip (catalog no. 4316567)
  - Thermo Fisher ultra-clear optical caps, strips of 8 (catalog no. AB-0866)
  - Thermo Fisher 96-well optical clear reaction plates (catalog no. A36924)
  - Thermo Fisher 96-well qPCR plate seals (catalog no. AB-1170)
- Applied Biosystems 7500 Fast Dx (0.1 mL) consumables.
  - Thermo Fisher optical 8-tube strip (catalog no. 4358293)
  - Thermo Fisher ultra-clear optical caps, strips of 8 (catalog no. 4323032)
  - Thermo Fisher 96-well optical reaction plates (catalog no. 4346906)
  - Thermo Fisher 96-well qPCR plate seals (catalog no. 4311971)
- Bio-Rad CFX96 Touch consumables.
  - Bio-Rad 8-tube PCR strips without caps (catalog no. TLS0851)
  - Bio-Rad Optical flat 8-cap strips for PCR tubes (catalog no. TCS0803)
  - Bio-Rad 96-well plates (catalog no. HSP9655)
  - Bio-Rad 96-well plate sealing film, optical (catalog no. MSB1001)
- Bio-Rad CFX384 Touch consumables.

  - Bio-Rad 384-well plates (catalog no. HSP3905)
  - Bio-Rad 384-well plate sealing film, optical (catalog no. MSB1001)
- BioGX pixl.16 real-time PCR instrument (BioGX catalog no. 650-016-PXL-R)
- BioGX pixl.16 consumables.
  - TempAssure® PCR 8-Tube Strips, Att. Optical Caps (BioGX catalog no. 010-280-ETS) or
  - EasyStrip™ Plus Tube Strip with Attached Ultra Clear Caps (Thermo Fisher catalog no. AB2005) or
  - Axygen® 0.2 mL Polypropylene PCR Tube Strips and attached Flat Cap Strips, 8 Tubes/Strip, (Corning catalog no. PCR-0208-AF-C)

WARNINGS AND PRECAUTIONS

- For research use only. Not intended for human or animal diagnostics use.
- If handling biological samples, including used Extraction Kits and PCR Cartridges, treat as if capable of transmitting infectious agents in accordance with safe laboratory procedures such as those described in CLSI Document M29[3] and in Biosafety in Microbiological and Biomedical Laboratories[4].
- BioGX REF: 450-082-LMP has been quality control tested only with the ABI QuantStudio™ 5, ABI 7500 Fast Dx, Bio-Rad CFX96 Touch™, Bio-Rad CFX384 Touch™ and BioGX pixl.16 real-time PCR platforms.
- Do not use the reagents if the protective pouches are open or torn upon arrival.
- Close reagent protective pouches promptly with the zip seal after each use. Remove any excess air in the pouches prior to sealing and store at 2-8 °C.
- Do not remove desiccant from the PCR master mix pouches.
- Do not use Sample-Ready™ master mix if the desiccant is not present or is broken inside the Sample-Ready™ master mix pouches.
- Do not mix reagents from different pouches and/or kits and/or lots.
- Do not use expired reagents and/or materials.
- Do not pipette by mouth.
- Do not smoke, drink, or eat in areas where samples or kits are being handled.
- Dispose of unused reagents and waste in accordance with country, federal, provincial, state, and local regulations.
- Use clean gloves when handling extraction kit components and PCR reagents.

STORAGE REQUIREMENTS AND RECOMMENDATIONS



Reagents are stable at a temperature range of 2-30°C during shipment for 5 days, but BioGX recommends long-term storage at 2-8°C. Reagents have been tested to demonstrate optimal performance when stored properly and consumed by the Manufacturer Recommended Use By Date. The end user may opt to extend the useful life for Research Use Only reagents upon completing their own performance validations. BioGX's guarantee of reagent integrity does not extend beyond the Manufacturer Recommended Use By Date when stored properly. Avoid exposing the reagents (lyophilized or rehydrated) to direct sunlight or long-term ambient lighting. Tightly reseal the pouch with unused reactions and immediately return to a refrigerator after opening. To mitigate reagent performance degradation from exposure to moisture, BioGX suggests using the entire contents of the opened pouch within 1 month; however, the user may choose to verify an extended working time > 1 month by performance testing with positive controls and an examination of the sample preparation control target.

SAMPLE TYPES UTILIZED FOR QUALITY CONTROL TESTING

-Urine (neat or boric acid preserved)
-Swab samples collected in Copan Universal Transport Media (UTM®) or Copan ESwab™

REAGENT OPTICAL CONFIGURATION

Table 1. Optical Channel Configuration for REF 450-082-LMP.

Optical Channel (Fluorophore Equivalent)	**Target**
FAM	Enterococcus faecalis
HEX	Unused
Texas Red	Enterococcus faecium
Cy5	RNase P
Cy5.5	Unused

QUALITY CONTROL AND EXTRACTION TESTING PARAMETERS

ABI, Bio-Rad and BioGX pixl.16 Platforms (BioGX REF: 450-082-LMP)

As a starting point, users can import and install a PCR run file onto:

1. Applied Biosystems QuantStudio™ 5 (Design & Analysis software version 1.5.1 or later)
2. ABI 7500 Fast Dx (SDS software version 1.4 or later)
3. Bio-Rad CFX96 Touch™ (Maestro software version 1.1 or later)
4. Bio-Rad CFX384 Touch™ (Maestro software version 1.1 or later)
5. BioGX pixl.16 real-time PCR platform (For Android® based software version 1.6.9 or later)

BioGX's most current ABI QuantStudio™ 5, ABI 7500 Fast Dx, Bio-Rad CFX96 Touch™, Bio-Rad CFX384 Touch™ and BioGX pixl.16 PCR run files utilized for quality control of this product can be obtained by sending an email to TS@biogx.com. Please refer to the Applied Biosystems QuantStudio™ 5 user manual[5] for uploading instructions. Please refer to the ABI 7500 Fast Dx user manual[6] for uploading instructions. Please refer to the Bio-Rad CFX96 Touch™ user manual[7] for uploading instructions. Please refer to the Bio-Rad CFX384 Touch™ user manual[7] for uploading instructions. Please refer to the BioGX pixl.16 user manual[8] for uploading instructions.

SAMPLE VOLUMES UTILIZED DURING QUALITY CONTROL TESTING

The end user may choose to validate a different pretreatment method or volume of sample to load other than the sample processing used by BioGX for QC testing as outlined below.

Urine collection (neat urine or boric acid preserved urine)

ABI, Bio-Rad and BioGX pixl.16 Platform (validated magnetic bead or silica column extraction kits) (REF: 450-082-LMP)

Thoroughly vortex the sample prior to processing. Follow manufacturer recommendations for extraction of appropriate sample volume. Transfer 5 µL of purified nucleic acid to master mix as described in Section: *BioGX Quality Control Test Setup Procedure for ABI, Bio-Rad and BioGX pixl.16 Platforms.*

Swab sample (Copan Universal Transport Media (UTM®) or Copan ESwab™)

ABI, Bio-Rad and BioGX pixl.16 Platform (validated magnetic bead or silica column extraction kits) (REF: 450-082-LMP)

Thoroughly vortex the sample prior to processing. Follow manufacturer recommendations for extraction of appropriate sample volume. Transfer 5 µL of purified nucleic acid to master mix as described in Section: *BioGX Quality Control Test Setup Procedure for ABI, Bio-Rad and BioGX pixl.16 Platforms.*

BioGX QUALITY CONTROL TEST SETUP PROCEDURE

ABI, Bio-Rad and BioGX pixl.16 Platforms Quality Control Test Setup

WEAR NITRILE GLOVES WHEN HANDLING LYOPHILIZED REAGENTS TO REDUCE THE GENERATION OF STATIC CHARGES. DO NOT USE LATEX GLOVES.

Assembly of BioGX Reagents

1. Prepare the appropriate number of 8-tube PCR strips, 96-well or 384-well PCR plates.
2. Transfer 40 µL of molecular grade water to one vial of lyophilized BioGX reagents. The rehydrated master mix is more than sufficient for 4 samples to be tested.

Note: 8-tube strips containing reagents **ARE NOT** compatible with ABI QuantStudio™ 5, ABI 7500 Fast Dx, Bio-Rad CFX96 Touch™, Bio-Rad CFX384 Touch™ and BioGX pixl.16 Platforms. Rehydrated master mix must be transferred to 8-tube PCR strips, 96-well or 384-well PCR plates compatible with appropriate instruments.

3. Mix by gently pipetting up and down. (IMPORTANT: Keep rehydrated master mix in a cold block or on ice if setup can not be completed within 20 minutes. If the rehydrated master mix cannot be used immediately, it can be capped and stored up to 24 hours at 2-8°C, protected from light).
4. Transfer 10 µL of rehydrated master mix to the bottom of 4 empty wells.
5. To each well containing 10 µL of rehydrated master mix, add 5 µL of extracted sample.
6. Affix the appropriate optical caps or optical plate seals.
7. Pulse spin the sealed 8-tube PCR strips, 96-well or 384-well PCR plates to mix and bring liquid to the bottom.
8. Load 8-tube PCR strips, 96-well or 384-well PCR plates into the real-time PCR platform and start the run. Avoid unnecessary delay once tubes/plates are loaded into the real-time PCR instrument.

ASSAY PERFORMANCE

All BioGX Research Use Only products are designed to detect 20 copies or less of the target nucleic acid per reaction.

INTERPRETATION OF RESULTS

Table 2. Multiplex PCR Results Interpretation for 450-082-LMP.

E.faecium	E.faecalis	RNase P	Interpretation
+	-	+/-	Enterococcus faecium POSITIVE
-	+	+/-	Enterococcus faecalis POSITIVE
-	-	+	Enterococcus faecium, Enterococcus faecalis NEGATIVE
-	-	-	Unresolved*

*Failed PCR due to inhibition, reagent failure or incorrect assembly of PCR reaction.

REFERENCES

1. Bartosch, Sabine, et al. "Characterization of bacterial communities in feces from healthy elderly volunteers and hospitalized elderly patients by using real-time PCR and effects of antibiotic treatment on the fecal microbiota." Applied and environmental microbiology 70.6 (2004): 3575-3581.
2. Li, Manshi, et al. "Identification of Enterococcus faecalis in a patient with urinary-tract infection based on metagenomic next-generation sequencing: a case report." BMC infectious diseases 20.1 (2020): 1-7.
3. Clinical and Laboratory Standards Institute. Protection of laboratory workers from occupationally acquired infections; Approved Guideline. Document M29 (Refer to the latest edition).
4. Centers for Disease Control and Prevention and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. Choosewood L.C. and Wilson D.E. (eds) (2009). HHS Publication No. (CDC) 21-1112.
5. QuantStudio™ Design and Analysis software User Guide, ThermoFisher Scientific, Waltham, Massachusetts, USA (Refer to the latest version).
6. Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument Instructions for Use (2012). Life Technologies Holdings Pte Ltd, Singapore. Publication Part Number 4406991 [Rev. E].
7. CFX96 Touch, CFX96 Touch Deep Well, CFX Connect, and CFX384 Touch Systems Instruction Manual (refer to the latest version), Bio-Rad Laboratories, Inc., Hercules, California, USA (Refer to the latest version).
8. BioGX pixl.16 Real-Time PCR Platform Instructions for Use (refer to the latest version), BioGX, Inc., Birmingham, Alabama, USA.

Please call BioGX or email info@biogx.com with any questions you may have regarding this product.

Rev. #	Effective Date	Summary of Changes
02	07 MAR 2025	Addition of product abbreviation code SRM11 (Sample-Ready Multiplex 11)
01	16 OCT 2024	Initial Release.

SYMBOLS

Symbol	Meaning	Symbol	Meaning
	Catalog number		Contains sufficient for <n> tests
	Research Use Only		Manufacturer
	Keep dry		Temperature limitation
	Consult instructions for use		Biological Risks

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