



YOUSEQ

BACILLUS SPP qPCR TEST KIT USER GUIDE

CAT NO.: YSL-qP-IC-Bac.SPP-100

100 reactions

With Internal Extraction control and Lyophilised MasterMix

VERSION 2.0

For Research Use Only



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INTENDED USE

This product is a qPCR test kit for the detection and quantification of Bacillus SPP (Bac.SPP) DNA in good quality nucleic acid samples from a variety of sources. It is designed to be used by trained users in a suitable molecular biology laboratory environment.

KIT CONTENTS

	Cap Colour	Volume
Bacillus SPP specific primer/probe (FAM probe)		110 µl
Internal extraction control primer/probe (VIC/HEX probe)		110 µl
Bacillus SPP positive control template		500 µl*
Internal extraction control DNA template		500 µl*
Lyophilised Tetra 2X qPCR MasterMix		1.1 ml*
MasterMix resuspension buffer		1.5 ml
Template resuspension buffer		1.5 ml
RNase/DNase free water		1.5 ml
ROX passive reference dye		10 µl

* Supplied lyophilised and requires resuspension before use, see resuspension step below for instructions

RESUSPENSION STEP

Resuspend the designated kit contents with the correct reagents as per the table below. Spin or gently tap the vial to ensure all the contents are at the bottom before opening.

After adding the resuspension reagent, pulse vortex the vial to ensure it is mixed well.

	Reagent	Volume
Bacillus SPP positive control template	Template resus. buffer	500 µl per vial
Internal extraction control DNA template	Template resus. buffer	500 µl per vial
Lyophilised Tetra 2X qPCR MasterMix	MasterMix resus. buffer	1.1 ml per vial

ROX (PLATFORM DEPENDENT)

ROX is required for platforms that use ROX as a passive reference. The table below outlines the hardware platforms that require the addition of ROX.

If ROX is required, dilute the ROX supplied according to the table below, then add 5µl to the fully resuspended Tetra MasterMix.

	Instruments	Step 1: Volume of water to add to ROX tube	Step 2: Add to MasterMix vial
High ROX Instruments	Applied Biosystems 7700, 7000, 7900, 7300, StepOne, StepOne Plus, and Roche capillary Lightcyclers 2.0	No Dilution Required	5 µl
Mid ROX Instruments	Stratagene MX	75 µl	5 µl
Low ROX Instruments	Applied Biosystems 7500 Platform, ViiA7 platforms, Quantstudio	130 µl	5 µl
All Other Machines		Not Required	Not Required

MATERIALS REQUIRED BUT NOT PROVIDED

DNA Extraction Kit – This qPCR test kit will work well with high quality DNA derived from any extraction kit with minimal PCR inhibitors present.

Pipettes, micro-centrifuge tubes and general laboratory equipment.

This qPCR kit will work on any instrument that detects VIC/HEX and FAM.

KIT SPECIFICITY

The YouSeq qPCR test kit for detection and quantification of Bacillus SPP is designed to have the broadest detection profile possible and detect all clinically relevant strains. The primers and probes have very high (>95%) homology with all reference data within the NCBI database.

The target genes for Bacillus SPP (LtaS gene, 16s gene & desR gene) have been demonstrated to have unique sequences making them ideal targets for highly specific detection of Bacillus SPP.

This kit will detect the following species: cereus, anthracis, thuringiensis, tropicus, pacificus, paranthracis, wiedmanni, bombysepticus, mobilis, toyonensismycooides, pseudomycooides, subtilis and atrophaeus.

If you require more specific data about the detection profile of the kit, please do not hesitate to contact our bioinformatics team: support@youseq.com

USE OF DNA INTERNAL CONTROL

Add 5 μl of the internal extraction control DNA into the extraction/lysis buffer for each sample that you are extracting. Make sure not to add this DNA directly in to your biological sample as this may cause degradation of the control DNA.

Continue DNA extraction as per the manufacturer's method.

qPCR BENCH SIDE PROTOCOL

Clean and decontaminate all work surfaces, pipettes, and other equipment prior to use to remove potentially contaminating nucleic acids.

REACTION SET UP

Combine the following reagents to create a final test reaction:

Component	Volume
Tetra 2X qPCR MasterMix	10 μl
Bacillus SPP specific primer/probe	1 μl
Internal extraction control primer/probe	1 μl
Extracted Sample DNA	8 μl
Final Volume	20 μl

NEGATIVE CONTROL

For a negative control reaction, repeat the reaction set up above replacing the sample DNA with DNase/RNase free water.



Please note: Make sure to seal the sample and negative control wells before proceeding to the positive control step.

POSITIVE CONTROL

In your designated post-PCR environment, perform a serial dilution of the positive control template to create a six-point standard curve.

1. Add 90 μl of template resuspension buffer into 5 tubes and label them 2, 3, 4, 5 and 6.
2. Pipette 10 μl of positive control template into tube 2.
3. Mix by pipetting up and down 5 times.
4. Change pipette tip and pipette 10 μl from tube 2 into tube 3.
5. Mix by pipetting up and down 5 times

Repeat steps 4 and 5 to complete the dilution process.

Combine the following reagents for each point of the standard curve:

Component	Volume
Tetra 2X qPCR MasterMix	10 μl
Bacillus SPP specific primer/probe	1 μl
Internal extraction control primer/probe	1 μl
Designated dilution of positive control	5 μl
DNase/RNase free water	3 μl
Final Volume	20 μl

The described standard curve provides a dynamic range as in the table below:

Tube No.	Copies of Target / rxn
1	1,000,000
2	100,000
3	10,000
4	1,000
5	100
6	10

Positive control standards 1-6



Other wells sealed to avoid contamination

qPCR AMPLIFICATION PROTOCOL

This YouSeq kit will work with any qPCR instrument capable of detecting FAM and VIC/HEX.

Run the following PCR protocol:

Please note: If using a qPCR machine that uses ROX as a passive reference, then the passive reference must be turned off or set to “none” indicating no passive reference.

	Temperature	Time
Hot Start	95°C	3 minutes
45 cycles	95°C	15 seconds
	60°C*	60 seconds

*Make sure to collect fluorogenic data through all the required target channels.

INTERPRETATION OF RESULTS

When analysing Sample Cq values, YouSeq recommends checking the threshold within the run file before interpreting the data. We would suggest setting the threshold to 10% of the relevant positive control End Point Fluorescence (EPF).

Positive Control

Firstly, check the positive control performance. It should amplify in a Cq range of approximately 18.5+/-2 for all targets. If the Cq range is not achieved, this would be a failed test and should be repeated.

Please note: The positive control in the kit is a representative sequence associated to the designs target region and does not contain the organism’s entire genome.

The positive control within the kit does not include the internal control sequence. Therefore, the positive control should not be expected to amplify in the internal control channel.

Negative Control

In ideal circumstances, the negative control well should deliver a flat line – negative result. However, it is not uncommon for background laboratory contamination to cause a very late signal. If this signal is ≥ 5 Cq values later than your sample signal, then it can be considered negative, and the result is viable.

If the negative is < 5 Cq later than the sample result, then the result is inconclusive, and the test should be repeated after potential sources of contamination have been removed.

Positive Samples

Samples that are positive for the target will deliver a defined “sigmoidal” amplification plot. Your qPCR instrument software will calculate a quantitative result for these signals by comparing the signal to the positive control standard curve. Results will be presented as target copy number.

Internal control

If your sample delivers a strong positive result for the target, then the internal extraction control is not required for data interpretation and can be ignored.

If your samples deliver a negative result, then the internal extraction control is useful to interpret the result. The Cq value from the internal extraction control will vary according to the amount of DNA in your sample. A late signal (Cq>28) indicates that only a small amount of host derived DNA was present in your sample. You may wish to repeat sample collection and then repeat the test in order to confirm the negative result.

Results interpretation:

	qPCR Signal		
Bacillus SPP (FAM probe)	≤35	-	-
Internal control (VIC/HEX probe)	+/-	+	-
Positive Control	+	+	+
Negative Control	-	-	-

Result

Positive result

Negative result.

Failed test.
Repeat required

PRODUCT SPECIFICATIONS

Storing your kit

Store at -20C from arrival. The qPCR kits shelf life is outlined as an expiry date on the pouch label. Once you have prepared the positive control it can be stored frozen.

Use good quality DNA

Poor quality input nucleic acid is the biggest cause of test failure. The kit will work well with any source of good quality DNA. Good quality is defined as DNA with high integrity (not degraded) and with low levels of inhibitors present.

Regulatory status

This product has been developed for Research Use Only and is not intended for diagnostic use. It should not be used for diagnosis of disease unless specifically approved by the regulatory authorities in the country of use.

Quality Control

In accordance with the YouSeq Ltd ISO EN 13485-certified Quality Management System, each lot of YouSeq Bacillus SPP qPCR test kit is tested against predetermined specifications to ensure consistent product quality. Design of the kit met our robust bioinformatic analysis requirements resulting in a clinically relevant detection profile based on available sequence information. The kit is periodically checked against newly available sequence information to remain clinically relevant.

Technical Assistance

For customer support, please contact:

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