YOUSEQ

LACTOBACILLUS JENSENII qPCR TEST KIT USER GUIDE

CAT NO.: YSL-qP-EC-L.jense-100

100 reactions
with Endogenous Control and Lyophilised MasterMix

VERSION 6.1

For research use only

YOUSEQ

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INTENDED USE

This product is a qPCR test kit for detection and quantification of Lactobacillus jensenii (L.jense) 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
L.jense specific primer/probe (FAM Probe)		110 µl
Endogenous control primer/probe (VIC/HEX Probe)		110 µl
ROX passive reference dye		10 µl
DNase/RNase free water		1.5 ml
Template resuspension buffer		1.5 ml
Lyophilised Tetra 2X qPCR MasterMix		1.1 ml*
MasterMix resuspension buffer		1.5 ml
L.jense positive control template		500 µl*

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

RESUSPENSION

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

After adding the resuspension reagent, pulse vortex the vials to ensure each is mixed well.

	Reagent	Volume
Lyophilised Tetra 2X qPCR MasterMix	MasterMix resus. buffer	1.1 ml
L.jense positive control template	Template resus. buffer	500 µl

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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.

qPCR instrument with minimum 2 colour detection (FAM and VIC/HEX).

Pipettes, micro centrifuge tubes and general laboratory equipment.

KIT SPECIFICITY

The YouSeq qPCR test kit for detection and quantification of Lactobacillus jensenii 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 genome region (threonine-tRNA ligase gene) has been demonstrated to have a unique sequence in this species making it an ideal target for highly specific detection of Lactobacillus jensenii.

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

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qPCR BENCH SIDE PROTOCOL

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

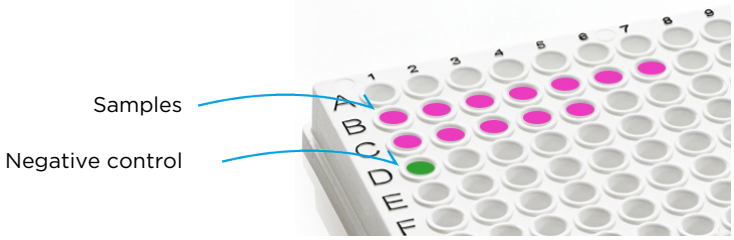
REACTION SET UP

Combine the following reagents to create a final test reaction:

Component	Volume
Tetra 2X qPCR MasterMix	10 µl
L.jense specific primer/probe	1 µl
Endogenous 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.

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POSITIVE CONTROL

In your designated post-PCR environment, perform a serial dilution of the positive control 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 into tube 2
3. Mix by pipetting up and down 5 times
4. Change pipette tip and pipette 10µl from tube 2 into tube 3
5. Mix by pipetting up and down 5 times

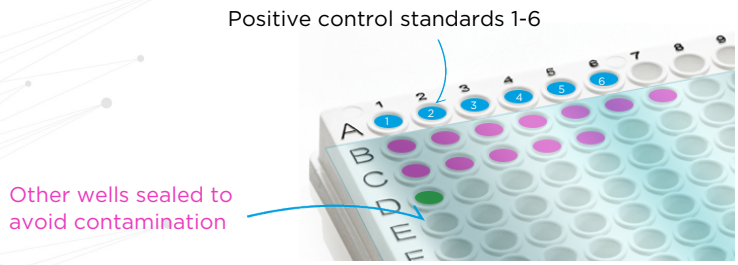
Repeat steps 4 and 5 to complete the dilution process.

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

Component	Volume
Tetra 2X qPCR MasterMix	10 µl
L.jense specific primer/probe	1 µl
Endogenous 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



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qPCR AMPLIFICATION PROTOCOL

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

Run the following PCR protocol:

	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 both the FAM and VIC/HEX channels during this step

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. The undiluted positive control should amplify in a Cq range of approximately 18.5 +/-2. If the Cq range is not achieved, this would be a failed test and should be repeated.

If running a positive control standard curve for a quantitative result, then an efficiency between 90% to 110% must be achieved. This will be automatically calculated by your analysis software. If it falls outside of this range, the run should be repeated with a freshly prepared standard curve.

Please note: The positive control in the kit is a representative sequence associated to the designs target region and does not contain the organisms entire genome.
The positive control within the kit does not include the endogenous control sequence. Therefore, the positive control should not be expected to amplify in the endogenous control channel.

Negative control

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

The test is valid if the following conditions are met:

	FAM Channel
Positive Control (Tube 1)	+
Negative Control	-

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INTERPRETATION OF RESULTS CONTINUED

Positive samples

Samples that are positive for Lactobacillus jensenii 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.

Endogenous control

If your sample delivers a strong positive result for Lactobacillus jensenii then the endogenous control is not required for data interpretation and can be ignored.
If your samples deliver a negative result, then the endogenous control is useful to interpret the result. The Cq value from the endogenous 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 at a glance:

	qPCR Signal		
Sample (FAM)	≤35	-	-
Endogenous Control (VIC/HEX)	+/-	+	-
Positive Control	+	+	+
Negative Control	-	-	-
Result	Positive result	Negative result	Failed test. Insufficient DNA

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PRODUCT SPECIFICATIONS

How sensitive is my kit?

The kit is suitable for the detection of Lactobacillus jensenii, across a wide dynamic range from 10^6 copies to less than 100 copies of the target in the PCR reaction.

Storing your kit

Store at -20°C from arrival. The qPCR kits shelf life is outlined as an expiry date on the pouch label.
Once you have prepared the positive control standard curve it can be stored frozen. However, if you observe a shift in Cq values in the standard curve over time a fresh standard curve should be prepared.

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 Lactobacillus jensenii 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:

e-mail: support@youseq.com
phone: +44 (0)333 577 6697

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