YOUSEQ

CLOSTRIDIUM BOTULINUM AND NEUROTOXIN GENES MULTIPLEX qPCR TEST KIT HANDBOOK

CAT NO.: YSL-qPX-IC-CbNg-100

100 reactions
With Internal Extraction Control and Lyophilised MasterMix

VERSION 2.0

For Research Use Only

YOUSEQ

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INTENDED USE

The YouSeq® product is a qPCR test kit for the detection of Clostridium botulinum and neurotoxin gene(s) 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
Clostridium botulinum and neurotoxin genes primer/probe mix: Clostridium botulinum primer/probe (FAM probe) Clostridium botulinum BoNT/B & BoNT/E primer/probe (VIC/HEX probe) Clostridium botulinum BoNT/A & BoNT/F primer/probe (ROX probe) Internal Extraction control (Cy5 probe)		100 µl
Clostridium botulinum and neurotoxin genes Positive control template		500 µl*
Tetra™ Lyophilised 2X qPCR MasterMix		2 x 1.1 ml*
MasterMix Resuspension Buffer (MMRB)		2 x 1.5 ml
Template Resuspension Buffer (TRB)		1.5 ml
RNase/DNase free water		1.5 ml

* Supplied lyophilised and requires resuspension, 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/tube to ensure all the contents are at the bottom before opening.

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

	Reagent	Volume
Clostridium botulinum and neurotoxin genes Positive control template	TRB	500 µl
Tetra™ Lyophilised 2X qPCR MasterMix	MMRB	1.1 ml per vial

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, ROX, Cy5 and FAM.

KIT SPECIFICITY

The YouSeq qPCR test kit for the detection of Clostridium botulinum and neurotoxin genes 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 Clostrodium botulinum (rsmH genes) and Clostridium botulinum neurotoxin genes (BoNT/B gene, BoNT/E gene, BoNT/A gene and BoNT/F gene) have been demonstrated to have distinctive sequences for highly specific detection of these targets.

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

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
Clostridium botulinum and neurotoxin genes primer/probe	1 µl
Extracted Sample DNA	9 µl
Final Volume	20 µl

NEGATIVE CONTROL

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

Please note: We advise sealing the sample and negative control wells before proceeding to the post-PCR environment (positive control step).

POSITIVE CONTROL

For a positive control, repeat the reaction set up above but replace the sample DNA with 9 µL of the resuspended positive control template supplied with the kit.

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qPCR AMPLIFICATION PROTOCOL

This YouSeq kit will work with any qPCR instrument capable of detecting FAM, VIC/HEX, ROX & Cy5.

Run the following qPCR 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 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. It should amplify in a Cq range of 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 supplied positive control is a sequence representative of the target region and does not contain the organism's entire genome.
The positive control does not include the control sequence and therefore, should not be expected to amplify in the Cy5 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.

The test is valid if the following conditions are met:

	ROX Channel	VIC/HEX Channel	FAM Channel
Positive Control	+	+	+
Negative Control	-	-	-

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Positive Samples

Samples that are positive for one of the targets will deliver a defined “sigmoidal” amplification plot.

Internal extraction 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 at a glance:

	qPCR Signal		
Clostridium botulinum and neurotoxin genes (Any Target Pathogen)	+	-	-
Internal extraction control: (Cy5)	+/-	+	-
Result	**Positive result based on specific channel amplification** Clostridium botulinum (FAM) BoNT/B & BoNT/E (VIC/HEX) BoNT/A & BoNT/F (ROX)	**Negative result**	**Failed test** Repeat required

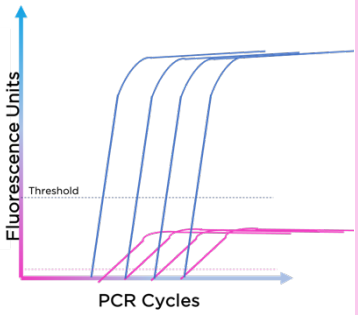
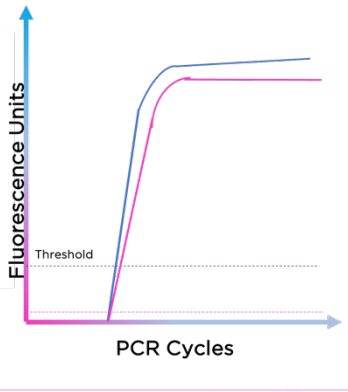
Positive results interpretation:

	qPCR Signal				
Clostridium botulinum (FAM)	+	+	+	-	-
Neurotoxin genes BoNT/B & BoNT/E (VIC/HEX)	-	+	-	+	-
Neurotoxin genes BoNT/A & BoNT/F (ROX)	-	-	+	-	+
Internal extraction control: (Cy5)	+/-	+/-	+/-	+/-	+/-
Result	**Positive result** Clostridium botulinum	**Positive result** Clostridium botulinum & Neurotoxin genes BoNT/B & BoNT/E	**Positive result** Clostridium botulinum & Neurotoxin genes BoNT/A & BoNT/F	**Positive result** Neurotoxin genes BoNT/B & BoNT/E of other clostridium species	**Invalid result** Repeat required

Coinfection

Positive signals could be observed in multiple channels on the occasion that a sample contains multiple BoNT genes.

MULTIPLEX TROUBLESHOOTING

	Trace	What can you see?	Cause	Action
1		One assay with greater end point fluorescence than another	Some fluorophores are brighter than others. Also, certain instruments detect different fluorophores with higher/lower efficiency	Analyse each channel individually so the Y-axis is correct for each fluorophore. or Analyse on logarithmic scale instead of linear scale
2		Amplification/ unusual Cq value in unexpected fluorescent channel. Cq value/curve shape very similar to adjacent fluorescent channel	'Bleed through' or 'cross talk' between channels. Amplification from one fluorescent channel has been mistakenly identified in its adjacent channel (e.g., FAM identified as HEX)	Ensure manufacturer recommends the dye combination used in this kit. Recalibrate qPCR instrument.

PRODUCT SPECIFICATIONS

Storing your kit

Store at -20C from arrival. The qPCR kits shelf life is outlines 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 Clostridium botulinum and neurotoxin genes Multiplex 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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Not available in all countries.