YOUSEQ

CLOSTRIDIUM BOTULINUM AND NEUROTOXIN GENES

MULTIPLEX qPCR TEST HANDBOOK

For Research Use Only. Not intended for diagnostic use.

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

100 reactions
With Internal Control and Lyophilised MasterMix

VERSION 4.0

YOUSEQ

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INTENDED USE

The qPCR test offers an efficient and user-friendly solution for the detection of Clostridium botulinum and neurotoxin gene(s) in extracted nucleic acid samples from a variety of sources. It is intended for use by trained professionals in a suitable molecular biology laboratory.

SPECIFICITY AND SENSITIVITY

Specificity

The YouSeq qPCR test for 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 typically have a ≥95% homology with all reference data used, from relevant, publicly available databases at the time of design.

The target genes outlined below, have been demonstrated to have distinctive sequences making them ideal targets for highly specific detection.
Clostridium botulinum: rsmH genes
Clostridium botulinum BoNT/B & BoNT/E: BoNT/B gene and BoNT/E gene
Clostridium botulinum BoNT/A & BoNT/F: BoNT/A gene and BoNT/F gene

For further information on the detection profile of the product, please do not hesitate to contact our team: support@youseq.com

Sensitivity

The qPCR test is suitable for the detection of these targets across a wide dynamic range. Under ideal PCR conditions the assay can detect less than 100 copies of the targets in the PCR reaction.

CONTENTS

omponent	Cap Colour	Volume
Multiplex primer/probe mix: C.botulinum (FAM) Neurotoxin B & E (VIC/HEX) Neurotoxin A & F (ROX) Internal Control (CY5)		100 µL
Lyophilised Tetra™ 2X qPCR MasterMix		1.1 mL*
MasterMix Resuspension Buffer (MMRB)		1.5 mL
C.botulinum & neurotoxins Positive Control (PTC)		500 µL*
Internal Control DNA Template		500 µL*
Template Resuspension Buffer (TRB)		1.5 mL
DNase/RNase Free Water		1.5 mL

* Supplied dried – requires resuspension. See instructions in resuspension section.

RECOMMENDED ADDITIONAL REAGENTS & MATERIALS

Nucleic acid extraction kit. – Internal Control DNA is to be included in the sample extraction. See 'Use of Internal Control DNA section below.

General laboratory equipment (pipettes, pipette tips, (micro)centrifuge tubes, compatible strip tubes/plates, plate seals, etc.)

qPCR instrument with channels to detect FAM, VIC/HEX, ROX and CY5.

BEST PRACTICE

Decontamination:

Before beginning laboratory work, thoroughly decontaminate any work surfaces and pipettes being used, to eliminate potential contamination.

General use and set-up:

All components should be fully defrosted with contents at the bottom of the tube before opening. To ensure contents are at the bottom, centrifuge or gently tap the tube. After use, reagents should be returned to the freezer.

Once any reagents are resuspended, mark the tick box on the tube for future reference. After this, or after combining reagents, the tube should be pulse vortexed to ensure it is mixed well.

It is advised to set up the tubes/plate and reaction mix on ice to minimise artefact formation, which may reduce sensitivity.

When preparing the qPCR reaction mix, it is recommended to incorporate an overage when calculating the total number of reactions to compensate for potential volume losses incurred during pipetting.

Set-up environments:

It is best practice to set up qPCR tubes/plates in two different environments - a clean (no template) lab and PCR (template) lab.

No Template Control(s) (NTC) and Positive Control(s) (PTC) should be included in every run. To reduce contamination, NTCs and samples can be set up and sealed in a clean lab before moving to the PCR lab.

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BENCH SIDE PROTOCOL

RESUSPENSION

Before first use, resuspend the designated component with the correct reagent and specified volume, as per the table below:

1. Add the resuspension reagent and pulse vortex the tube to ensure each is mixed well.

Component	Reagent	Volume	Location
Internal Control DNA Template	TRB	500 µL	Extraction Lab
Lyophilised Tetra™ 2X qPCR MasterMix	MMRB	1.1 mL	Clean Lab
C.botulinum & neurotoxins Positive Control (PTC)	TRB	500 µL	PCR lab

USE OF INTERNAL CONTROL DNA

1. At the relevant step of the extraction protocol, pause and add 5 µL of the resuspended Internal Control [DNA] into the extraction/lysis buffer for each sample that is to be extracted.

Please note: Do not add this Internal Control [DNA] directly into the biological sample as this may cause degradation of the control [DNA].

2. Continue [nucleic acid] extraction as per the manufacturer's instructions.

qPCR REACTION SET-UP

1. Retrieve the required components and appropriate plasticware for [qPCR] reaction set-up.
2. In an appropriately sized (micro)centrifuge tube, combine the following reagents to create a reaction mix that will cover all required wells (e.g. sample(s), NTCs and PTCs).

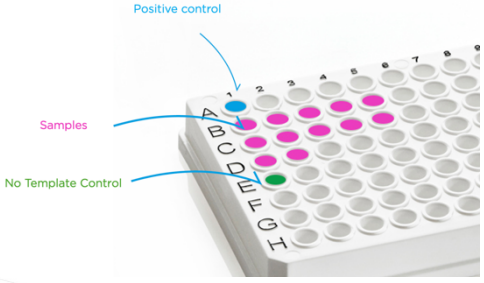
Please note: When calculating required reactions, include an overage to allow for volume loss during pipetting

Component	Volume (per reaction)
Tetra™ 2X [qPCR] MasterMix	10 µL
[C.botulinum & neurotoxins primer/probe mix]	1 µL
Reaction mix volume	11 µL

3. Mix the combined reagents by briefly vortexing or inverting.
4. Dispense 11 µL of the reaction mix into all required tubes/wells.
5. For the NTC(s), add 9 µL of DNase/RNase Free Water into required tube/well(s).
6. For each extracted sample, add 9 µL into required tube/well(s).

Please note: It is best practice to seal the NTC(s) and sample tube/wells before proceeding to the positive control step.

7. For the positive control(s), add 9 µL of the resuspended PTC, into the required tube/well(s).
8. Seal the tube(s)/plate.
9. Briefly vortex the sealed tube(s)/plate, then spin in a centrifuge to ensure all



reagents are fully resuspended and at the bottom of the tube(s)/wells before proceeding.

qPCR AMPLIFICATION PROTOCOL

1. Load the tubes/plate onto the qPCR instrument and set up the qPCR protocol following the table below.

2. Set the total reaction volume to 20 µL.

Please note: If using a qPCR instrument that uses ROX as a passive reference, ensure the passive reference is turned off or set to "none" before starting the run.

Temperature	Time	Number of Cycles
95°C	3 minutes	-
95°C	15 seconds	x 45
60°C 📷	60 seconds	

📷 Collect fluorogenic data through FAM, VIC/HEX, ROX and CY5 channels during this step.

3. Start the run.

INTERPRETATION OF RESULTS – OVERVIEW

If using single threshold analysis – YouSeq recommends setting the threshold at 10% of the End Point Fluorescence (EPF) for each channel.

- For the Target channel, use the PTC EPF to set the threshold.
- For the Internal Control, use the average EPF from samples to set the threshold.

Results interpretation:

Reaction Type		qPCR Signal	
Positive control		18 ± 2	18 ± 2
No template control		-	-
Sample	Internal Control: (CY5)	≤ 31	≤ 31
	Target(s): (FAM or VIC/HEX or ROX)	+	-
Result		**Positive result: target specific** See table below	**Negative result**

Positive results interpretation:

Positive Result	qPCR Signal				
Clostridium botulinum (FAM)	+	+	+	+	-
Neurotoxin genes BoNT/B & BoNT/E (VIC/HEX)	-	+	-	+	+
Neurotoxin genes BoNT/A & BoNT/F (ROX)	-	-	+	+	-
Result	**Positive result** C. botulinum	**Positive result** C. botulinum & BoNT/B.BoNT/E	**Positive result** C. botulinum &	**Positive result** C. botulinum, BoNT/B.BoNT/E &	**Positive result** BoNT/B.BoNT/E of other Clostridium

INTERPRETATION OF RESULTS – CONTROLS EXPLAINED

Positive control

The PTC should amplify in a Cq range of 18 ± 2 for each target. If this Cq range is not achieved, the sample test result for the associated target is invalid and should be repeated.

Please note: The positive control is a sequence representative of the target regions and does not contain the organism's entire genome. The positive control does not include the Internal Control sequence and should not be expected to amplify in the CY5 channel.

No template control

The NTC well(s) should be negative, with no amplification.

Please note: Background laboratory contamination can result in a very late signal in NTC wells. If the NTC has amplification, comparison to the sample test result is necessary:

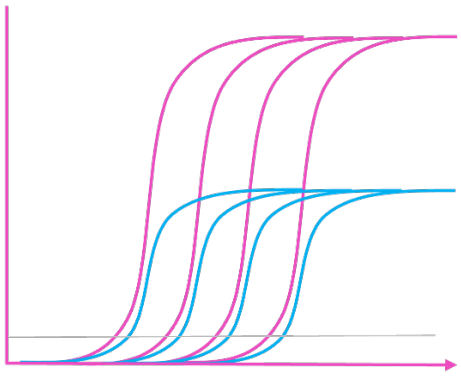
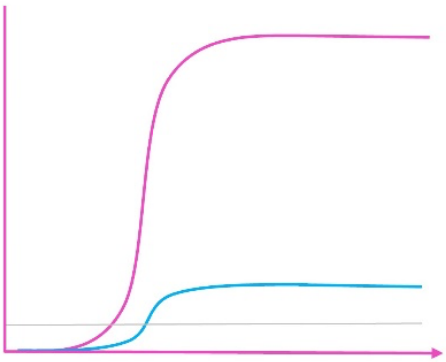
- If the NTC is ≥5 Cq later than the sample signal, it can be considered negative, and the sample test result is valid.
- If the NTC is <5 Cq later, the sample test result is invalid – the test should be repeated after potential sources of contamination have been removed.

Internal control

Detection of the Internal Control is through the CY5 channel. This gives information about the efficiency of the nucleic acid extraction. Cq values ≤ 31 indicate a successful extraction has taken place. If the signal is later than this, repeating the nucleic acid extraction is advised.

Please note: If the sample delivers a strong positive result for the target of interest, then amplification of the Internal Control may be affected and may appear later. In this case, lack of Internal Control amplification is acceptable, and the sample test result is valid.

MULTIPLEX TROUBLESHOOTING

	1	2
Trace		
What can you see?	Different fluorescence levels between Targets	Unexpected low level of fluorescence, similar to amplification from a different channel, within the same well
Cause	Fluorescence depends on the assay design and the channel e.g. FAM channel is typically the brightest	'Bleed-through' between channels; Amplification from one channel has been incorrectly assigned to a different channel e.g. fluorescence in FAM 'bleeding-through' to VIC/HEX
Action	Analyse each channel individually so the Y-axis is appropriate for that assay	Ensure instrument is compatible with the dye(s) used in this test and/or recalibrate qPCR instrument

PRODUCT SPECIFICATIONS

Storage

Store at -20°C from arrival. The product's shelf life is outlined as an expiry date on the pouch label.

Suitable input material

This qPCR test will work well with any source of good quality nucleic acid. Good quality is defined as nucleic acid with high integrity (not degraded). Poor quality input nucleic acid is a leading cause of test failure.

Regulatory status

This product has been developed for Research Use Only (RUO) and is not intended for diagnostic use. It should not be used for diagnosis of disease or infection 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 qPCR Test is tested against predetermined specifications to ensure consistent product quality. The primers/probe(s) typically demonstrate ≥95% *in silico* specificity to their intended target and are periodically checked against newly available sequence information to maintain their detection profile.

Technical Assistance

For customer support, please contact:

e-mail: support@youseq.com
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