Xpert qDetect *Vibrio* spp.

#GDK04.0100 (100 rxns)
(FOR RESEARCH ONLY)

Product: The genus *Vibrio* consists of a large number of species of motile Gram-negative bacteria that are commonly found in salt water environments. Several species are a major cause of human foodborne illness worldwide. Human infections with these facultative anaerobic pathogens are associated with the consumption of undercooked seafood and contaminated drinking water.

V. cholerae and *V. parahaemolyticus* are two species of particular interest as they can induce clinical syndromes ranging from mild gastroenteritis and vibrosis to life-threatening sepsis and cholera.

Traditional microbiological detection and confirmation methods are long established and well developed but typically require 4-5 days in order to obtain results. This qPCR Detection Kit provides a fast, sensitive and reliable method for the detection of *V. cholerae* and *V. parahaemolyticus* based on qPCR using precisely designed specific primers and FAM-labeled Taqman® probes, requiring less than 2 days. This immense time reduction allows taking appropriate action, if needed, much sooner.

This kit is compatible with instruments equipped with FAM and ROX channels. The detection limit is approximately 500fg of *Vibrio* DNA allowing the detection of, after enrichment, as little as 1-10 cells per 25g of food sample, with a specificity of 100%.

Applications: Detection of *Vibrio* spp. in food and animal feeding stuffs (following bacterial enrichment and subsequent DNA extraction (see: "prior to use")).

Contents: The qPCR Detection Kit (#GDK04.0100) for *Vibrio* spp. contains sufficient reagents for 100 qPCR Reactions (e.g. 100rxns of *V. cholerae* or 100rxns of *V. parahaemolyticus* or 50rxns each, etc.)

Component	GDK04.0100
Vspp Mix A	2x 840 µl
Vspp Mix B for *V. cholerae*	210 µl
Vspp Mix C for *V. parahaemolyticus*	210 µl
Positive Control (Vspp+)	70 µl
Negative Control (Vspp-)	70 µl

Note: This product does not include reagents and other materials required for DNA extraction.

Properties:
Fast, Easy and Reliable
Low limit of Detection
Validated according to ISO 22118:2011
100% Specificity
Compatible with instruments equipped with **FAM** and **ROX** channels

Storage: -20ºC and protected from light for at least 1 year. Minimize repeated freeze/thawing, consider preparation of aliquots.

Prior to use:

Safety Considerations
V. cholerae and *V. parahaemolyticus* are pathogenic to humans, hence isolation and identification must be carried out by trained laboratory personnel, in a properly equipped laboratory. Care must be taken in the sterilization and disposal of all test materials. All procedures must be performed in the designated area of the laboratory. Disposable gloves must be worn during all procedures. All relevant National and Local Regulations must be met.

Laboratory Set-Up
In order to prevent erroneous results due to contamination and/or degradation of DNA, it is strongly recommended to have dedicated materials, equipment and area for the DNA extraction, separated from the preparation of PCR procedures. Workflow in the laboratory should be unidirectional, starting with DNA isolation and moving to preparation of PCR amplification and finally to (q)PCR and Detection. Moreover, all molecular biology procedures, including DNA extraction, require qualified personnel to prevent risk of erroneous results and/or inadequate interpretation of results.

Bacterial Enrichment
This kit is for the detection of DNA from *Vibrio* spp. present in total DNA previously purified from a bacterial pre-enrichment culture of a defined sample of food or animal feed. Representative food samples should be prepared conform standard procedures and under GLP conditions [1]. It is highly recommended to carry out the pre-enrichment according to ISO 21872-1:2017[2] or BAM (Chapter 9).[3]

DNA extraction
Following incubation, cells are harvested from 1ml of the enriched culture by centrifugation at 10,000g-12,000g for 5 minutes. After washing the pellet with 0.9% Sodium Chloride (NaCl), add 200µl of Lysis Buffer (e.g. 10mM Tris-HCl pH 8.0 containing 1mM EDTA and 0.1% Tween®20) to the pellet. Resuspend by vortexing or pipetting up and down in the tube. Incubate at 95°C-100°C for 10-15 minutes using a water bath or heating block. Centrifuge 10,000g-12,000g for 2 minutes and transfer the cleared supernatant to a new microtube. Use 2µl of the supernatant as template DNA sample in the qDetect protocol.
Alternatively, DNA is extracted from the sodium chloride-washed pellet using an appropriate kit for the extraction of DNA from Gram-negative bacteria (not included) using the manufacturer´s instructions. The obtained highly-purified DNA, free of possible PCR inhibitors, can be used immediately or stored at -20°C for up to several years for future analysis. Use 2µl of the eluate as template DNA sample in the qDetect protocol.

References

1. Public Health England (2016) Preparation of samples and dilutions, plating and sub-culture. Microbiology Services. Food, Water & Environmental Microbiology Standard Method FNES26 (F2); Version 2.

2. Microbiology of the food chain - Horizontal method for the determination of *Vibrio* spp. - Part 1: Detection of potentially enteropathogenic *Vibrio parahaemolyticus, Vibrio cholerae* and *Vibrio vulnificus* (ISO 21872-1:2017)

3. FDA´s Bacteriological Analytical Manual, Chapter 9 (revised May 2004). An online edition of BAM Chapter 9 is available at https://www.fda.gov/food/laboratory-methods-food/bam.chapter-9-vibrio

qDetect - Basic Protocol

Unambiguous detection of *Vibrio* spp. (and of the internal control) requires a suitable calibration of both FAM and ROX channel. Please refer to the manufacturer´s instruction of the real time PCR cycler.

1. **Mix for each qPCR reaction**:

Component	Volume
Vspp Mix A	16 µl
Vspp Mix B	2 µl

In order to minimize risk of contamination, reagent loss and improve pipetting accuracy, we recommend to prepare a mastermix for multiple samples (N), always including a negative control, and a positive control, by mixing all components (N+2), except template DNA (nor control DNA), dividing the mixture equally into each PCR tube (18 µl each), briefly spin tubes (or tap down) and then add 2 µl template DNA or control DNA directly in the mixture.

2. **Set-up qPCR cycling**:

Nº cycles	Temp	Time	Acquisition
1x	50ºC	2 min	No
1x	95ºC	5 min	No
40x	95ºC	30 sec	No
	60ºC	30 sec	Yes
	72ºC	30 sec	No

After an initial cycle of 2 min at 50ºC and 5 min at 95ºC (Enzyme activation and denaturation of template), cycle 40 times for 30 seconds at 95ºC, 30 seconds at 60ºC and 30 seconds at 72ºC. Acquire data for the detection of *Vibrio* spp. on the **FAM** channel. Probe to detect specific amplification of the internal control, which is included in Vspp Mix A and which is amplified simultaneously with the target DNA, should be detected in the **ROX** channel.

Results

Controls
In order to validate the assay, controls must have the following results. If the signal of one of the controls does not match, the whole experiment, including all samples, must be repeated.

Control	FAM channel	ROX channel
Negative Control	Ct=N/A	positive
Positive Control	positive	unimportant*

N/A = Not applicable (signal below threshold).
*unimportant: is expected to be positive, however, if negative but other controls match expected results, this makes no difference

Samples
For each sample, there are 4 possible outcomes, as summarized in the table below.

FAM channel	ROX channel	Result
positive	positive	positive
positive	Ct=N/A	positive
Ct=N/A	positive	negative
Ct=N/A	Ct=N/A	inhibition*

*) in case both *Vibrio* (FAM channel) and Internal Control (ROX channel) have signals below threshold, but all the controls resulted in signals as expected, the sample must be retested, as the qPCR reaction was inhibited. Inhibition often is the result of a too high DNA concentration and therefore it is recommended that retesting should be carried out with a 10-fold dilution of the original DNA sample.