Bile-Tolerant Gramm-Negative Bacterial (BTGN) Count Probe qPCR Detection

Catalog Number	Kit Size
TBS42042-100	100
TBS42042-200	200

DESCRIPTION

Bile-tolerant Gram-negative (BTGN) bacteria are a diverse group of microorganisms equipped with a protective outer membrane that enables them to survive harsh environments, including the acidic conditions of the human stomach and exposure to bile salts. This group includes critical pathogens and indicators such as *E. coli*, *Salmonella* spp., *Shigella* spp., *Klebsiella* spp., and *Pseudomonas aeruginosa*. Because their presence signals poor hygiene or potential health risks, the monitoring and determination of BTGN are strictly regulated across the food safety, cannabis, and pharmaceutical industries.

To meet these compliance standards, **Tribioscience's BTGN Probe qPCR Detection Kit** offers a rapid method designed to screen for BTGN contamination. Utilizing real-time quantitative polymerase chain reaction (qPCR) alongside dual-labeled fluorogenic probes, the assay delivers highly sensitive quantitative and qualitative results. The assay kit is an all-in-one version, including all essential components—optimized qPCR Master Mix, Primer-Probe Mix, and both positive and negative controls, ensuring a streamlined workflow and straightforward result interpretation.

PRINCIPLE

The **Tribioscience BTGN Probe qPCR Detection Kit** utilizes real-time quantitative PCR (qPCR) for the highly sensitive and specific amplification of bile-tolerant Gram-negative bacteria with target-specific fluorescent probes. As the PCR product accumulates, there is an increased fluorescent signal from the bound probes. Monitoring the fluorescence intensities during the PCR run allows the detection of the accumulating PCR product in real time.

KEY FEATURES

- High sensitivity and specificity for BTGN bacteria.
- High efficiency: Optimal systemic conditions for PCR amplifications.
- Streamlined protocol: Just add DNA template, and water.
- No cross-reactivity with other species.

APPLICATIONS

- Detect bile-tolerant Gram-negative bacteria in leaves, roots, flowers, and other agricultural samples.
- The kit can be used in Bio-Rad, Thermofisher, Agilent, Roche LightCycler, Qiagen, and other multicolor real-time qPCR systems with FAM and HEX fluorescence channels.

KIT CONTENTS

Name	100x rxn	200x rxn
qPCR Super Mix	0.9 mL	1.8 mL
Primer-probe Mix	0.5 mL	1.0 mL
Positive Control DNA	10 µL	20 µL
Negative Control DNA	60 µL	120 µL

The BTGN Probe is labeled with FAM, and the PCR internal control is labeled with HEX.

STORAGE CONDITION

The kit is shipped on ice and stored at -20°C for long-term storage. The shelf life is 12 months after receipt.

Note: TSB medium (TBS8065), Dead Bacterial DNA Eraser (TBS6039), and Microbial DNA Magnetic Extraction Kit (TBS6025) are separately purchased if clients need them.

SAMPLE PREPARATION AND BACTERIAL CULTURE

1. Weigh out 1g of sample and put into the labeled Whirl-pak bag. (Note: if processing multiple samples, gloves should be changed between each to ensure there is no cross contamination of the samples during the weighting process.).
2. Add 9mL of TSB medium (TBS8065) to Whirl-Pak bag. (Note: Sample is diluted 10 times, DF=10)
3. Close the whirl-Pak bag. Mix the sample and the TSB medium for at least 1minute.
4. Incubate the Whirl-Pak bag or conical tube at 37˚C for 16 - 24 hours.
5. Prepare and label a 1.5mL tube with the sample name and date.
6. Take 1 mL culture liquid without plant debris and others from the bag and put into a labeled 1.5mL.
7. Centrifuge for 5 min at 10,000 rpm at RT, and carefully discard supernatant, keep the cell pellet in the tube for DNA extraction.

OPTION: DEAD BACTERIAL DNA REMOVAL FROM CELL PELLET FOR ALIVE BACTERIAL DNA EXTRACTION

Suitable methods for dead bacterial DNA removal can be used. We recommend Tribioscience Dead Bacterial DNA Eraser Kit (TBS6039) for this procedure. This kit was validated for dead bacterial removal). The Procedures are below:

1. Add 10 µL Dead Bacterial DNA Eraser to bacterial sample enrichment, mix gently.
2. Incubate at 37°C for 30 minutes.
3. Inactivation: Incubate at 90°C in heating block for 10 min.
4. Cool down into ice.
5. Spin down the solution in the tube bottom.
6. The treated sample can be used for DNA extraction.

DNA EXTRACTION (Recommended for TBS6025)

1. Resuspend the cell pellet completely in 200 µL of Lysis Buffer1(Note: If the Lysis Buffer has any turbid, please warm up it at 37˚C to dissolve it). Vortex vigorously for 1min to mix completely. Incubate at room temperature (RT) for 5 min or longer.
2. Add 200 µL of chloroform, mix vigorously.
3. Centrifuge the lysate at 13,000 rpm at RT for 5 min.
4. Take supernatant (about 200 µL) to 96well extraction plate (Note: the 96 well plate is labeled before adding samples).

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5. Add 200 µL of Binding Buffer 2 into the sample well the plate, mix for several time with Pipette (Note: The Binding Buffer 2 must be resuspended completely before use).
6. Incubate the extraction plate at RT on the bench for 5min.
7. Put the extraction plate onto the magnetic plate for 5min, then remove as much of 400 µL of the supernatant as possible (Note: do not disturb or aspirate the beads).
8. Washing 2 times with 70% ethanol (EtOH): Add 400 µL of 70% EtOH to sample wells on the extraction plate still on the magnet plate, then incubate for 1min. Remove all EtOH. Repeat this step again, aspirate all EtOH.
9. Dry the beads in the extraction plate at RT for 15 min.
10. Add 50 uL Elute Buffer 3 and resuspend the beads with pipette. Incubate the plate for 1 min or longer on the bench. Then put the extraction plate back to the magnetic plate, and incubate for at least 1 min.
11. Transfer the eluent to a new extraction plate with sample label or keep the same extraction plate, and store at -20°C for further applications, like PCR detections.

QPCR PROCEDURES

1. Prepare PCR Master Mix as Below:

Reagents	1 Reaction
qPCR Mix	8 µL
Primer-probe Mix	4 µL
Water	3 µL
Total	15 µL/well

Note: Prepare enough master mix for your samples plus two controls (positive and NTC). Add 10% overage to the master mix components to account for pipetting and dead volumes.

2. Transfer 5 µL of each sample, or 5 µL Positive Control, or 5 µL Negative Control into indicated wells of a qPCR plate in duplicate manner.
3. Transfer 15 µL of freshly prepared qPCR Master Mix to each well and slowly tip mix. Avoid adding bubbles to the mixture.
4. Seal plate tightly, spin in plate centrifuge and load on qPCR machine.
5. Set up qPCR cycling parameters as below:

Step	PCR Amplification		
	Pre-denature	CYCLE (40x cycles)	
		Denature	Anneal/ Extend
Temperature	94°C	94°C	58°C
Time	1 min	10 sec	1 min

6. Start the run.
7. When the run is complete, the plate can be discarded.
8. Proceed with data analysis.

DATA ANALYSIS

Qualitative Analysis

1. **BTGN Positive (Fam):** Sample Ct ≤ 37, Positive control and Negative and Blank controls normal amplification.
2. **BTGN Negative (Fam):** Sample Ct > 35 or non-amplification, Positive control and Negative and Blank controls normal amplification.
3. PCR internal control (Hex) is positive signal in all samples, positive and negative controls. The internal control amplification indicates a normal PCR amplification. Otherwise, the PCR reaction may be inhibited.
4. Repeat Reaction: If one of the control reactions is not normal, PCR reaction fails and should be repeated.

Quantitative Analysis

The quantitative analysis is based on the Ct value for calculation of Colony-Forming Unit (CFU)/g in samples. The equation below to calculate the CFU/g:

CFU/g =4.5109 x 10^(10-0.2918Ct) x DF

Ct: qPCR amplification Threshold value
DF: Sample dilution Factor in sample preparation step.

RELATIVE PRODUCTS

TBS6025: Microbial DNA Magnetic Extraction
TBS42025: 4-In-1Aspergillus qPCR
TBS42027: STEC qPCR
TBS42028: Salmonella qPCR
TBS42029: STEC and Salmonella Multiple qPCR
TBS42030: Mycoplasma Detection qPCR
TBS42031: Listeria Monocytogen qPCR
TBS42032: Listeria Genus qPCR
TBS42033: Bacillus Cereus qPCR
TBS42034: Bacillus Species qPCR
TBS42050: Pseudomonas Aeruginosa and Staphylococcus Aureus Detection qPCR
TBS42051: Escherichia coli (E. Coli) – Salmonella qPCR
TBS42021: Aspergillus Flavus qPCR
TBS42022: Aspergillus Fumigatus qPCR
TBS42023: Aspergillus Niger qPCR
TBS42024: Aspergillus Terreus qPCR
TBS42067: 5-Plex Aspergillus qPCR