Xpert qDetect *E.coli* Serotypes
(O157,O26,O111,O103,O145)

#GDK06.0250 (50 rxns each)

(FOR RESEARCH ONLY)

Product: Most *Escherichia coli* strains are harmless; however, some can cause serious food poisoning. Enteric *E.coli* that is responsible for foodborne disease can present different virulence determinants, corresponding to different pathotypes[1], including the verocytotoxigenic *E.coli* (VTEC), comprising the enterohaemorrhagic *E.coli* (EHEC) and the enteropathogenic *E.coli* (EPEC). VTEC (or STEC) are characterized by the production of verocytotoxins (Vtx), also known as Shiga-like toxins (Stx), which are encoded by *vtx1* or *vtx2* genes. EHEC (including O157:H7 and O157:H-) are a subset of VTEC, which in addition to Vtx-encoding genes harbour the intimin-coding gene *eae* that cause attaching and effacing lesions in hosts, and are recognized as major pathogens of foodborne disease in humans. The EPEC synthesize intimin but not Vtx. Whereas VTEC comprises of a large number of serotypes, those associated with the more severe forms of disease, such as haemolytic uremic syndrome (HUS), include O157, O126, O111, O103, and O145[2].

Traditional microbiological detection and confirmation methods are long established and well developed but typically require many days in order to obtain results. This qPCR Detection Kit provides a fast, sensitive and reliable method for the detection of HUSEC *E.coli* serotypes on qPCR using specific FAM-labeled 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 50-500fg of *E.coli* 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 *E.coli* serotypes (O157, O26, O111, O103, O145) in food samples (following bacterial enrichment and subsequent DNA extraction (see: "prior to use")).

Contents: The qPCR Detection Kit (#GDK06.0100) for contains sufficient reagents for 250 qPCR reactions. (50 rxns for each of O157, O26, O111, O103 and O145 serotype).

Component	GDK06.0100
Ecs Mix A	5x 840 µl
O157 Mix	105 µl
O26 Mix	105 µl
O111 Mix	105 µl
O103 Mix	105 µl
O145 Mix	105 µl
Positive Control (HUSEC+)	175 µl
Negative Control (HUSEC-)	175 µl

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

Properties: Fast, Easy and Reliable
Low limit of Detection, 100% Specificity
Validated according to ISO 22118:2011
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
Some *E.coli* are pathogenic to humans, hence isolation and identification must be carried out by trained laboratory personnel, in a properly equipped laboratory. Most activities can be carried out in a Containment Level 2 laboratory, yet only laboratories with containment level 3 should test samples for STEC/VTEC O157, O26, O111, O103 and O145 as they are Hazard Group 3 pathogens. 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 the presence of *E.coli* serotypes O157, O26, O111, O103 and/or O145 in foodstuff. First a bacterial enrichment cultures from 25g of food should be prepared. For this, representative food samples should be prepared conform standard procedures and under GLP conditions [3]. It is highly recommended to carry out the pre-enrichment according to ISO 16654:2001[4] or BAM (Chapter 4)[5]**.** Subsequently, bacterial DNA should be extracted, as described below, and finally the DNA should be screened for the presence of DNA from the *E.coli* serotypes O157, O26, O111, O103 and/or O145, as desired.
Instead of screening directly for DNA of the serotypes O157, O26, O111, O103 and/or O145, often one screens the bacterial DNA first for the presence of *E.coli* VTEC/STEC pathotype, (e.g. using GRiSP´s GDK02.0150 Xpert E.coli (EPEC, VTEC and EHEC)) and only in case of positive results subsequently screens individual colonies for serotypes O157, O26, O111, O103 or O145.

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.

For the screening of individual colonies, resuspend a colony in 100µl of Lysis Buffer (e.g. 10mM Tris-HCl pH 8.0 containing 1mM EDTA and 0.1% Tween®20) and 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.

References

1. ESFA Panel on Biological Hazards (BIOHAZ) (2013). Scientific opinion on VTEC-seropathotype and scientific criteria regarding pathogenicity assessment ESFA Journal 11(4):3138
2. Microbiology of food and animal feed – Real-time polymerase chain reaction (PCR)-based method for the detection of food-borne pathogens – Horizontal method for the detection of Shiga toxin-producing *Escherichia coli* (STEC) and the determination of O157, O111, O26, O103 and O145 serogroups (ISO/TS 13136:2012)
3. 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.
4. Microbiology of food and animal feeding stuffs - Horizontal method for the detection of Escherichia coli O157 (ISO 16654:2001)
5. FDA´s Bacteriological Analytical Manual, Chapter 4 (revised October 2000). An online edition of BAM Chapter 7 is available at https://www.fda.gov/food/laboratory-methods-food/bam-chapter-4-enumeration-escherichia-coli-and-coliform-bacteria

qDetect - Basic Protocol

Unambiguous detection of *E.coli* genes (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
Ecs Mix A	16 µl
Serotype Specific Mix*	2 µl

**) For detection of O157 serotype use O157 mix, for O26 use O26 Mix, for O111 use O111 Mix, for O103 use O103 Mix and for O145 use O145 Mix*

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 *E.coli* serotypes on the **FAM** channel. Probe to detect specific amplification of the internal control, which is included in Ecs 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 (HUSEC-)	Ct=N/A	positive
Positive Control (HUSEC+)	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.

E.coli O157, O26, O111, O103 **or** O145 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 *E.coli* serotype (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.