

# Instructions for Use

## Life Science Kits & Assays



**innuDETECT Campylobacter spp. Assay**

## 1 Product specifications

| Starting material       | Isolated total DNA        |
|-------------------------|---------------------------|
| Time of detection       | ~ 60 minutes              |
| qPCR detection channels | FAM (Target) and HEX (IC) |
| Sensitivity             | Up to 5 DNA copies/PCR    |

Detection of DNA derived from sample material using an extraction kit suitable to isolate bacterial DNA. Please make sure that the common quality requirements for DNA samples are achieved.

## 2 Intended use

The innuDETECT *Campylobacter* spp. Assay has been designed for detection of DNA derived from genus *Campylobacter* using TaqMan® principle.

*Campylobacter* is a gram-negative, rod-shaped bacterium and belongs to the most important pathogens inducing food-borne infections in humans. The most important infectious species are *C. jejuni*, *C. coli* and *C. lari*. A contamination may occur in food like poultry and poultry related products (excluding eggs), unpasteurized milk, drinking water or pets.

The Assay detects the *Campylobacter* genus specific sequence of acyl-[acyl-carrier-protein]-UDP-N-acetylglucosamine O-acyltransferase (IpxA) gene.

The assay includes an Internal Control (IC) that can be used as amplification control if added to the PCR reaction. If added to the Lysis Buffer the IC can also be utilized to check the DNA-extraction method used.

The assay is intended for research use only.

### 3 Product and order number

| Name                                | Amount | Order-no.       |
|-------------------------------------|--------|-----------------|
| innuDETECT Campylobacter spp. Assay | 24 rxn | 845-IDF-0024024 |
| innuDETECT Campylobacter spp. Assay | 96 rxn | 845-IDF-0024096 |






### 4 Storage conditions

The Assay is delivered at ambient temperature.

Store the innuDETECT Campylobacter spp. Assay at -22 °C to -18 °C, except the innuDRY qPCR MasterMix Probe that should be stored before dissolving at 4 °C –8 °C.

When stored as recommended, the kit is stable until the expiration date printed on the label on the kit box.

### 5 Delivered components

| Components                   |  | ∇ 24   | ∇ 96     |
|------------------------------|--|--------|----------|
| Primer/Probe Mix Cam.spp. IC |  | 75 µl  | 300 µl   |
| innuDRY qPCR MasterMix Probe |  | 1      | 1        |
| Resuspension Buffer Probe    |  | 300 µl | 1.1 ml   |
| Internal Control             |  | 1      | 1        |
| PCR-grade H <sub>2</sub> O   |  | 2 ml   | 2 x 2 ml |

## 6 Safety precautions

The assay shall only be handled by educated personal in a laboratory environment. The compliance with the specified procedure is absolutely mandatory when performing this assay.

Reagents should be stored in their original containers at the indicated temperatures. Do not replace individual components with those from different batches or test assays. Note the indicated expiration dates.

Do not eat, drink or smoke while performing the assay.

Wear protective clothing and safety gloves.

All samples and test materials should be handled and disposed of as infectious material, in accordance with regulatory requirements.

Reagent containers that have not come in contact with potentially infectious material may be disposed of along with ordinary laboratory waste.

Store the reagents used for performing PCR separately from DNA templates and amplification products.

## 7 Reagent preparation

### 7.1 Internal Control

Dissolve the lyophilized Internal Control (IC) by adding 1.25 ml of PCR-grade H<sub>2</sub>O and mix thoroughly.

To use the IC as an amplification control, add 1 µl of IC to each PCR reaction.

Alternatively, the IC can be added to the qPCR reaction mix in an amount of 1 µl/reaction. NOTE: in this case the No Template Control (NTC) must also be positive for IC.

To improve the sensitivity of the test for samples with a very low target amount, reduce the IC content up to 1:10.

To use the IC as an extraction control, add to the Lysis Buffer/Sample Mix the amount of IC which is 1/10 of the final elution volume (see according DNA isolation instruction manual). Use the co-amplification of spiked IC to observe the relative loss of DNA during the extraction procedure

### 7.2 2x MasterMix

The 2x MasterMix must be prepared before starting the PCR setup and can be stored at -22 °C to -18 °C.

Add 250 µl for 24 rxn assay or 1 ml for 96 rxn assay of Resuspension Buffer Probe to the innuDRY qPCR MasterMix Probe tube. Vortex carefully and centrifuge the tube to collect the liquid on the bottom.

## 8 Real-Time PCR (qPCR)

### 8.1 Preparation of reaction batches

Determine the total number of required qPCR reactions considering also at least one NTC.

The composition of the qPCR reaction mix for one sample is shown in the table below. Prepare the qPCR reaction mix for the number of samples needed (including NTC).

Add qPCR reaction mix to PCR stripe or plate. In a second step add samples to the qPCR reaction mix in order to avoid the cross contamination.

| Reagent                                     | Volume (1 rxn)                      |
|---|-------------------------------------|
| 2x MasterMix                                | 10 $\mu$ l                          |
| Primer/Probe Mix Cam.spp. IC                | 3 $\mu$ l                           |
| IC (if used as amplification IC)            | 1 $\mu$ l                           |
| Sample (PCR-grade H <sub>2</sub> O for NTC) | $\leq$ 2 $\mu$ g DNA, max 5 $\mu$ l |
| PCR-grade H <sub>2</sub> O                  | Fill up to 20 $\mu$ l               |

Seal the PCR stripe or plate with an appropriate sealing film (PP) and/or cap; place tubes in the Real-Time PCR Cycler and close the lid.

### 8.2 Real-Time PCR conditions

For basic information regarding the setup and programming of the different Real-Time PCR Cycler, please refer to the manual of the respective instrument.

## Interpretation of results

Program the Real-Time PCR Cyclers as indicated in the table below and start the program.

| Step | Cycles | Profile                 | Temperature | Retention time |
|------|--------|-------------------------|-------------|----------------|
| 1    | 1      | Initial denaturation    | 95 °C       | 120 s          |
| 2    | 40     | Denaturation            | 95 °C       | 10 s           |
|      |        | Annealing / Elongation* | 60 °C       | 45 s           |

\* Data acquisition: Fluorescence Detection (FAM; HEX)

## 9 Interpretation of results

Please refer to the following table to identify the signal pattern that matches to the obtained signals. It is strongly recommended to run at least one No Template Control (NTC) for each experiment.

The Ct value of IC can vary (or even disappear) in dependence of DNA quality and intensity of FAM signal.

| FAM | HEX | Sample         | Valid | Recommended interpretation                                 |
|-----|-----|----------------|-------|--|
| +   | (+) | NTC            | no    | Contamination of PCR chemicals with target or (and) IC DNA |
| -   | (+) | NTC            | yes   | No contamination   |
| +/- | +   | Unknown Sample | yes   | Positive or negative for the target                        |
| -   | -   | Unknown Sample | no    | PCR reaction and/or DNA isolation failed                   |
| ++  | -   | Unknown Sample | yes   | The sample is strong positive for the target               |

## 10 Related products

| Product                                 | Order Number    |
|---|-----------------|
| innuDETECT Salmonella spp. Assay        | 845-IDF-0022... |
| innuDETECT Salmonella enterica Assay    | 845-IDF-0023... |
| innuDETECT Listeria spp. Assay          | 845-IDF-0029... |
| innuDETECT Listeria monocytogenes Assay | 845-IDF-0021... |
| innuDETECT Shiga Toxin 1 Assay          | 845-IDF-0025... |
| innuDETECT Shiga Toxin 2 Assay          | 845-IDF-0026... |
| innuDETECT E.coli O157 Assay            | 845-IDF-0027... |
| innuDETECT E.coli O104 Assay            | 845-IDF-0028... |

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