

Instructions for Use

Life Science Kits & Assays



innuDETECT *Yersinia enterocolitica* Assay

1 Product specifications

Starting material	Isolated total DNA
Time of detection	~ 90 minutes
qPCR detection channels	FAM (<i>Yersinia enterocolitica</i>) HEX (Internal Control)

The Assay detects DNA isolated from sample material using an extraction kit able to isolate total bacterial DNA. A suitable DNA isolation kit from Analytik Jena is highly recommended. Please make sure that the common quality requirements for DNA samples are achieved.

2 Intended use

The innuDETECT *Yersinia enterocolitica* Assay has been designed to detect the DNA derived from *Yersinia enterocolitica* using TaqMan principle.

Y. enterocolitica is a gram-negative bacillus-shaped bacterium. *Y. enterocolitica* infection causes gastroenteritis, which is an animal-borne disease occurring in humans, as well as in a wide array of animals such as cattle, deer, pigs, and birds.

The Assay detects the *Y. enterocolitica* specific sequence of *yst*-gene (detected in FAM-channel).

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 used DNA extraction method.

The assay is intended for research use only.

3 Product and order number

Name	Amount	Order-no.
innuDETECT Yersinia enterocolitica Assay	24 rxn	845-IDF-0032024
innuDETECT Yersinia enterocolitica Assay	96 rxn	845-IDF-0032096

4 Storage conditions

The kit is shipped at ambient temperature. Upon arrival store the innuDETECT Yersinia enterocolitica Assay at -22 °C to -18 °C, except the innuDRY qPCR MasterMix Probe that before dissolving should be stored 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 Safety precautions

The assay shall only be handled by educated personal in a laboratory environment. The compliance with the specified procedure is absolute 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.

Delivered components

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.

6 Delivered components

Components		∑ 24	∑ 96
Primer/Probe Mix Y.entero IC		75 µl	300 µl
innuDRY qPCR MasterMix Probe		1 (25 rxn)	1 (100 rxn)
Resuspension Buffer Probe		300 µl	1.1 ml
Internal Control		1	1
PCR-grade H ₂ O		2 ml	2 x 2 ml

7 Reagent preparation

7.1 Internal Control

Dissolve the lyophilized Internal Control (IC) by adding 1.25 ml of PCR-grade H₂O and mixing thoroughly.

To use the IC as an amplification control, add the IC to the qPCR reaction mix in an amount of 0,1 µl per reaction. NOTE: the No Template Control (NTC) must also be positive for IC.

To use the IC as an extraction control, add to the Lysis Buffer/Sample Mix an amount of IC which is 1/10 of the final

elution volume (see corresponding DNA isolation manual). To observe the relative loss of DNA during the extraction procedure, perform the amplification of 1 µl of 1:10 diluted IC added to the NTC. This corresponds to 100% DNA extraction efficiency.

7.2 2x qPCR MasterMix Probe

The 2x innuDRY qPCR MasterMix Probe 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. Incubate the tube in a thermal shaker for 20 minutes at 25°C and 550 rpm. Spin down the tube to collect the liquid on the bottom.

8 Real-Time PCR (qPCR)

8.1 Preparation of Reaction Mix

Determine the total number of required qPCR reactions considering your samples and at least one NTC.

The composition of the qPCR Reaction Mix per single reaction (1 rxn) is shown in the table below. Prepare the qPCR Reaction Mix for the number of reactions needed with a 10 % surplus volume to allow for pipetting error.

Reagent	Volume (1 rxn)
2x qPCR MasterMix Probe	10 µl
Primer/Probe Mix Yersinia/IC	3 µl
IC, diluted 1:10 (if used as amplification IC)	1 µl
Sample (PCR-grade H ₂ O for NTC)	≤ 2 µg DNA, max 5 µl
PCR-grade H ₂ O	Fill up to 20 µl

To avoid the cross contamination first aliquot the qPCR Reaction Mix to PCR stripe or plate and then add samples to the qPCR reaction mix.

8.2 Real-Time PCR Conditions

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

Program the Real-Time PCR device 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	45	Denaturation	95 °C	15 s
		Annealing / Elongation*	55 °C	60 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.

If the sample is strongly positive for *Yersinia enterocolitica*, in rare cases it is possible that the IC is negative.

Interpretation of results

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 for <i>Yersinia enterocolitica</i>
-	+	Unknown Sample	yes	Negative for <i>Yersinia enterocolitica</i>
-	-	Unknown Sample	no	PCR reaction and/or DNA isolation failed
+	-	Unknown Sample	yes	The sample is strong positive for the target

Publication No.: HB_IDF-0032_e_200117

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