

Instructions for Use

Life Science Kits & Assays



innuDETECT E. coli O104 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 *E. coli* O104 Assay has been designed for detection of DNA derived from *E. coli* O104 gene cluster using TaqMan® principle.

E. coli O104 is an enterohemorrhagic serotype of the bacterium *E. coli* and a cause of illness, typically through consumption of contaminated food. Infection may lead to hemorrhagic diarrhea, and to kidney failure. Transmission is via the fecal-oral route, and most illnesses have occurred after ingestion of contaminated raw green leafy vegetables and undercooked meat.

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 E. coli O104 Assay	24 rxn	845-IDF-0028024
innuDETECT E. coli O104 Assay	96 rxn	845-IDF-0028096






4 Storage conditions

The Assay is delivered at ambient temperature.

Store the innuDETECT E. coli O104 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 O104 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 ₂ 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 H₂O 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 μ l
Primer/Probe Mix O104 IC	3 μ l
IC (if used as amplification IC)	1 μ l
Sample (PCR-grade H ₂ O for NTC)	\leq 2 μ g DNA, max 5 μ l
PCR-grade H ₂ O	Fill up to 20 μ 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 Campylobacter spp. Assay	845-IDF-0024...
innuDETECT Shiga Toxin 1 Assay	845-IDF-0025...
innuDETECT Shiga Toxin 2 Assay	845-IDF-0026...
innuDETECT E.coli O157 Assay	845-IDF-0027...

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Manufacturer / Distributor / Publisher:

IST Innuscreen GmbH
Robert-Rössle-Strasse 10
13125 Berlin · Germany

Telefon +49 (0)30 9489 3380
Telefax +49 (0)30 9489 3381
info.innu@ist-ag.com