Instructions for Use
Life Science Kits & Assays

innuDETECT Bacteria Quantification Assay

Innuscreen GmbH

1 Product specifications

Starting material	Isolated DNA/Bacterial Standard DNA
Time of detection	~ 60 minutes
qPCR detection channels	FAM (Target) and HEX (IC)
Sensitivity	Dependent on the background of the Negative Control

The Assay detects DNA isolated from sample material using an extraction kit able to isolate total bacterial DNA. Please make sure that the common quality requirements for DNA samples are achieved.

2 Intended use

The innuDETECT Bacteria Quantification Assay is a tool enabling semi-quantification of bacterial DNA from any species of bacteria. The Kit includes a standard DNA with a definite amount of bacterial DNA and a primer/probe set for a TaqMan probe-based detection of all kinds of bacteria.

The target sequence is the 16S ribosomal RNA 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 efficiency of the used DNA extraction method.

The assay is intended for research use only.

3 Product and order number

Name	Amount	Order no.
innuDETECT Bacteria Quantification Assay	24 rxn	845-IDF-0031024
innuDETECT Bacteria Quantification Assay	96 rxn	845-IDF-0031096

4 Storage conditions

The Assay is delivered at ambient temperature.

The kit is shipped at ambient temperature. Upon arrival store the innuDETECT Bacteria Quantification 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 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.

6 Delivered components

Components		Σ 24	Σ 96
Standard DNA (10^7 copies/µl)		30 µl	30 µl
Primer/Probe Mix GK IC		75 µl	300 µl
innuDRY qPCR MasterMix Probe		1x25 rxn	1x100 rxn
Resuspension Buffer Probe		300 µl	1.1 ml
Internal Control		1	1
PCR-grade H_2O		2 x 2 ml	3 x 2 ml

7 Reagent preparation

NOTE
The environment as well as the DNA-extraction and PCR chemicals are not free from bacteria and bacterial DNA. Therefore, to monitor the bacterial background of your preparation we recommend carrying a "background control sample" **(BC)**: a DNA extraction from PCR-grade H_2O instead of a sample.

7.1. Standard Dilutions

Prepare a 10-fold serial dilution of the Standard DNA (STD) in PCR-grade H_2O. For the final template copies number given in molecules per µl see the following table:

STD	Volume H_2O	Volume STD	Concentration
STD 1	45 µl	5 µl	10^6 copies/µl
STD 2	45 µl	5 µl from STD 1	10^5 copies/µl
STD 3	45 µl	5 µl from STD 2	10^4 copies/µl
STD 4	45 µl	5 µl from STD 3	10^3 copies/µl
STD 5	45 µl	5 µl from STD 4	10^2 copies/µl

7.2. Internal Control

Dissolve the lyophilized Internal Control (IC) by adding 1.25 ml of PCR-grade H_2O 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 1µl of 1:10 diluted IC added to the NTC. This corresponds to 100% DNA extraction efficiency.

7.3. 2x PCR MasterMix Probe

The 2x PCR 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) respectively 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 dawn 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, standard dilutions, at least one NTC and/or the Background Control (BC).

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.

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.

NOTE
Minimum of 3 10-fold standard dilutions are necessary for a generation of the standard curve!

Don't forget to add the Internal Control to the Standard Dilution Samples and to the negative controls, if it was not previously added to the Reaction Mix!

Reagent	Volume (1 rxn)
2x PCR MasterMix Probe	10 µl
Primer/Probe Mix GK IC	3 µl
IC (if used as amplification IC)	1 µl
Sample (PCR-grade H_2O for NTC) or PC Standard DNA	≤ 2 µg DNA, max 5 µl 1 µl
PCR-grade H_2O	Fill up to 20 µl

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.

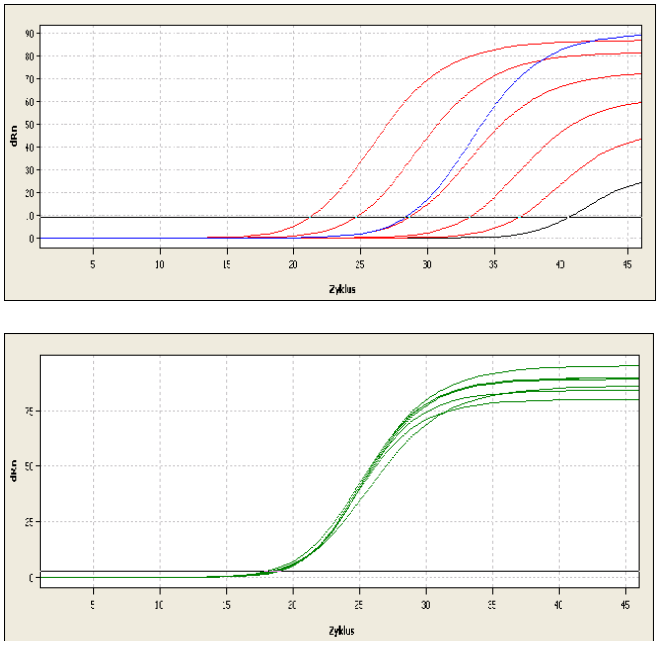
Program the Real-Time PCR Cycler as indicated in the table below and start the program.

Step	Cycles	Profile	Temperature	Retention time
1	1	Initial denaturation	95 °C	120 s
2	40	Denaturation	95 °C	10 s
		Annealing / Elongation*	56 °C	45 s

* Data acquisition: Fluorescence Detection (FAM; HEX)

9 Interpretation of results

- All samples with Ct values lower than the negative controls and/or than the Background Control sample (see the note to chapter 7.) are positive. If the Ct of the sample is equal or higher than Ct of the negative control, then the bacterial load of the sample is lower, than the chemicals background (BC) and therefore it cannot be calculated.
- Generally, if you define your dilution series as standards with known target amount, all Real-Time Cyclers can automatically calculate the amount of target DNA in unknown samples. An application example is given below.



Sample	Ct	Copies/rxn
STD-1	21,5	10^6
STD-2	24,8	10^5
STD-3	28,4	10^4
STD-4	32,6	10^3
STD-5	36,1	10^2
BC	40,2	
Sample	28,2	2930

Red: 1:10 dilution series (FAM)
Black: BC
Blue: unknown sample (FAM)
Green: Internal Control (HEX)

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