

Instructions for Use

Life Science Kits & Assays



innuDETECT Internal Control DNA/RNA Assay

1 Product specifications

The innuDETECT Internal Control DNA/RNA Assay was developed to ensure correct sample preparation procedure by the use of an artificially designed internal control (IC). The assay can be used to evaluate nucleic acid extraction and to verify the absence of inhibition during reverse transcription and/or amplification. The IC is detected by Real-time PCR in most commercially available multi channel real time cyclers (table 1):

Cycler	Channel for IC
qTOWER ³ (Analytik Jena)	Color module 2 or 3
LightCycler [®] (Roche)	Detection channel 2 (HEX, VIC)
CFX (BioRad)	All Channels module (HEX, DY-549, Yakima Yellow, JOE)
StepOne (Thermo Scientific)	JOE, HEX, Yakima Yellow
Rotor-Gene Q (QIAGEN)	Yellow Channel (JOE, VIC, HEX, etc.)

Both IC and target gene can be analyzed simultaneously when the chosen fluorophores can be measured in different channels.

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.

2 Product and order number





Name	Amount	Order-no.
innuDETECT Internal Control DNA Assay	100 rxn	845-ID-0006100
innuDETECT Internal Control RNA Assay	100 rxn	845-ID-0007100
innuDETECT Internal Control DNA/RNA Assay	100 rxn	845-ID-0008100

3 Storage conditions

Store the innuDETECT Internal Control DNA/RNA Assays at -22 °C to -18 °C.

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

4 Delivered components

Components		100
Primer/Probe Mix DNA		33 µl
<i>and / or (depending of order)</i>		
Primer/ Probe Mix RNA		33 µl
Internal Control DNA and/or RNA (Carrier Mix)		lyophilized, add 1.25 ml PCR-grade H ₂ O
PCR-grade H ₂ O		2 ml

5 Reagent preparation

Dilute the freeze-dried IC in 1.25 ml PCR-grade H₂O before use. Aliquot the diluted IC and store at -22 °C to -18 °C. Avoid frequent freeze-and-thaw cycles.

6 Detection methods

7.1 Extraction control for DNA / RNA

Certain Analytik Jena Kits contain already the IC. If you use those Kits follow the appropriate instructions of the kit. If you use a different extraction kit, take note of the following instructions:

The IC has to be added to the sample/lysis buffer mixture. The volume of the IC depends on the elution volume you finally want to use. It should represent 10 % of the elution volume. For instance, if DNA/RNA will be eluted in 100 µl, add 10µl IC to the sample/lysis buffer mixture.

Important Notes:

- As the IC contains carrier DNA/RNA check its compatibility with kits which also contain a step where carrier DNA/RNA is added. Maybe this step should be omitted.
- Do not add the IC directly to the original sample! Make sure that lysis buffer has always been added before!
- Depending on quality and method of isolation, the yield of IC can vary or even fail.

The isolated nucleic acids now can be used for subsequent tests.

7.2 Amplification control for DNA

Attention! If you already added the IC during the extraction, add IC only to the negative control sample of your PCR reaction, but not to the PCR MasterMix.

The MasterMix is prepared as usual with your target specific primers and probe. Additionally add the following components:

Reagent	Volume (1 rxn)
Primer/Probe Mix DNA	0.3 μ l
Internal Control	0.1 μ l
Total	0.4 μ l

7.3 Amplification control for RNA

Attention! If you already added the IC during the extraction, add IC only to the negative control sample of your PCR reaction, but not to the Reverse Transcription or PCR MasterMix.

For OneStep Real-time PCR the master mix is prepared as usual with target specific primers and probe. Additionally add the following components:

Reagent	Volume (1 rxn)
Primer/Probe Mix DNA	0.3 μ l
Internal Control	0.1 μ l
Total	0.4 μ l

For TwoStep real-time PCR add 1.0 μ l of IC per sample to the Reverse Transcription MasterMix and use Random Primers to assure a correct cDNA synthesis of the IC. To the master mix for Real-time PCR only 0.3 μ l of Primer/Probe Mix RNA must be added.

7.4 Real-Time PCR conditions

For the detection of the IC use the following settings:

IC reporter dye channel: see table in *Product specifications*

Annealing temperature real-time PCR: 59°C +/- 2°C
(depends on your target)

These settings can be used under standard and rapid cycling conditions.

7 Interpretation of results

Sample	Target	IC	Interpretation
Unknown Sample	+	+	Target is in sample
Unknown Sample	+	-	Strong positive target in sample
Unknown Sample	-	+	No target in sample
Unknown Sample	-	-	Run is invalid due to incorrect sample preparation, PCR setup or inhibition in the DNA sample
NTC	-	+	No contamination
NTC	-	-	Run is invalid due to incorrect PCR setup
NTC	+	(+)	Run is invalid due to contamination

8 Application examples

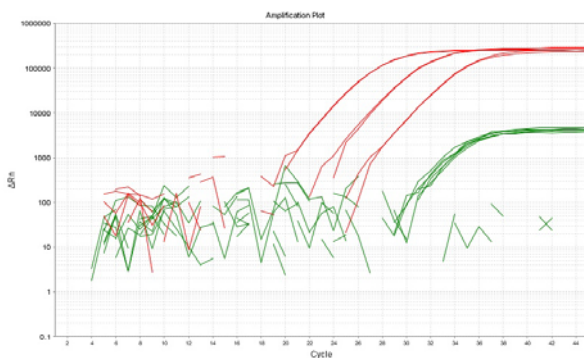
9.1 Amplification control for DNA

The experiment was performed with target DNA (green channel) and IC DNA (yellow channel). The Primer/Probe mix DNA was used.

9.2 Amplification control for RNA

The experiment was performed with RNA target (green channel) and IC RNA (yellow channel). The Primer/Probe Mix RNA was used.

Amplification control for **DNA**



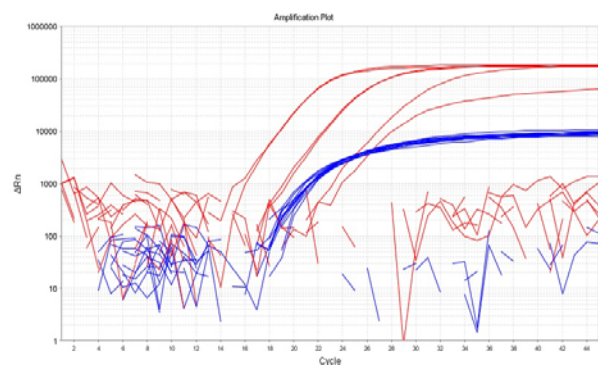
Target 1 (red):

Stx2 gene from 10^6 , 10^5 , 10^4 EHEC cells in the starting sample

Target 2 (green):

Internal control DNA

Amplification control for **RNA**



Target 1 (red):

Different samples of H5N1-Virus HA-gene

Target 2 (blue):

Internal control RNA

9 Related products

Product	Order Number
innuPREP Virus DNA Kit	845-KS-4600
innuPREP Virus DNA/RNA Kit	845-KS-4800
innuPREP Virus RNA Kit	845-KS-4700
innuMIX qPCR MasterMix Probe	845-AS-1200100
innuTaq Hot-A DNA Polymerase	845-EZ-3000500
50x inNucleotide Mix	845-AS-9000100

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