INgene q Lawsonia intracellularis

R.11.LAW.K.5/50 *50 Reactions*

R.11.LAW.K.5/100 *100 Reactions*

qPCR kit for the detection of **Lawsonia intracellularis** in biological samples. *Real Time PCR (TaqMan technology).*

1.INTRODUCTION AND INTENDED USE

INgene q Lawsonia intracellularis is a real-time PCR kit suited for the detection and/or quantification of **Lawsonia intracellularis** (**L. intracellularis**) **DNA** in biological samples of clinical interest from **Swine** in a simple way, with high sensitivity and specificity.

The assay is suitable for the following sample types: **faeces, rectal swabs, intestine** and strains/vaccines.

For research use only

2.PRINCIPLE OF THE ASSAY

INgene q Lawsonia intracellularis is an assay for amplification and detection of the **L. intracellularis** genetic material based on the polymerase chain reaction (PCR) and the use of hydrolysis probes (TaqMan type) for the fluorescent detection of amplified products. The kit contains all necessary reagents and enzymes to amplify and simultaneously detect **L. intracellularis** genetic material in extracted nucleic acids samples. The reagents are included in a ready to use assay mix, that is in optimal concentrations to detect (and optionally quantify) the genetic material of **L. intracellularis**, simply by adding the extracted nucleic acids of the problem sample. This kit contains specific primers and a probe labelled with FAM fluorochrome for the detection of **L. intracellularis** at 530 nm. In addition, the assay mix includes an endogenous control (EC) that is amplified with specific primers and detected with a probe labelled with HEX/VIC, allowing identification of false negatives due to PCR inhibition.

The kit includes a **L. intracellularis** synthetic positive control for detection and optionally perform relative/absolute quantification. This control could be used to generate a standard curve in order to correlate the number of pathogen copies with the quantification cycle threshold (Cq) value.

The kit has been validated in the following PCR platforms: QuantStudio 5 Dx System from Applied Biosystems, and CFX96™ Real-Time PCR System from Bio-Rad. It is compatible with other thermocyclers, if they have the appropriate fluorescence channels, but Cq values may vary among them.

3.KIT COMPOSITION

COMPONENTS	DESCRIPTION	Nº VIALS	VOLUMEN/VIAL
INgene q Lawsonia intracellularis ASSAY MIX TUBE	Buffers, enzyme, dNTPs, primers/probes for **L. intracellularis** and EC detection	1 or 2 [1]	750 µl
PC10 [2] POSITIVE CONTROL	Lyophilized specific synthetic control	1 [3]	Lyophilized
Water	Nuclease free water	1	1 ml

[1] 1 vial for 50 reactions format and 2 for 100 reactions format.

[2] Reconstitute the Positive Control (PC) as described below:

1. Spin briefly the PC tube and reconstitute with 250 µl of nuclease free water.

2. To facilitate resuspension keep the tube for 10 min at room temperature.
3. Vortex gently the tube before use.
4. Store reconstituted PC at ≤-18ºC.

[3] See the Certificate of Analysis (CoA) for copy number.

4.CONSERVATION OF THE KIT COMPONENTS

All components have to be stored at ≤-18ºC immediately after arrival and until the moment of use and protected from light. Reagents should not be used after the expiration date. See the Certificate of Analysis.

5.MATERIALS AND EQUIPMENT REQUIRED BUT NOT SUPPLIED

- Powder-free disposable gloves
- Microcentrifuge
- Tube shaker
- Real-time thermocycler
- Micropipettes (2-1000 µl) and sterile pipette tips (with filter)
- Ice bucket or cooling block
- Sterile microtubes
- qPCR plates or tubes with optical caps/sealing compatible with the thermocycler

6.WORKING GUIDELINES AND PRECAUTIONS TO AVOID CONTAMINATION

The following working guidelines should be carefully observed:

- Use nuclease-free/molecular grade materials and sterile filtered tips.
- Ensure refrigeration and good homogenisation of the kit components once defrosted.
- Avoid repeated cycles of freezing and defrosting of the kit components, as it can reduce kit performance.

Special precautions to avoid ambient or cross-contaminations that could cause false positive results:

- Use at least two separate working areas with dedicated material and unidirectional workflow: (1) Isolation of the nucleic acids and (2) Amplification/detection area.
- The positive PCR control should be stored separately from the rest of kit reagents. During the qPCR setup, manipulate and dispense the positive control in the last place, avoiding any contact with the other kit components.

7.SAMPLES COLLECTION AND TRANSPORT

Samples should be collected and handled carefully to avoid contamination and should arrive in the testing laboratory as soon as possible, following national regulations for their transport. The samples must be cooled from the moment they are obtained and during the transport until they are processed. Processing needs to occur within 24 hours after obtaining the samples. In case a longer time is envisaged, freezing of samples is recommended.

8.NUCLEIC ACIDS EXTRACTION

Any extraction procedure which yields good-quality of the extracted material could be used. Parallel to the extraction of the genetic material of the samples, it is advised to perform a mock-extraction, in which the biological sample is replaced by nuclease free water, to check possible contamination during the extraction procedure.

Some recommended kits:

a) Manual Nucleic Acids extraction:

- QIAamp DNA Blood Mini Kit (QIAGEN, Inc.)
- QIAamp Viral RNA Mini Kit (QIAGEN, Inc.)
- DNeasy Blood & Tissue Kit (QIAGEN, Inc.)
- RNeasy PowerLyzer Tissue & Cells Kit (QIAGEN, Inc.)
- SpeedTools Total RNA extraction Kit (Biotools, S.A.)

b) Automated DNA extraction:

- MagMAX CORE™ Nucleic Acid Purification Kit (Thermo Fisher Scientific)

- MagNA Pure 24 Total NA Isolation Kit

with their respectives automatic extraction robots (KingFisher™Flex; MagNA Pure 24 System) following the manufacturer´s instructions.

CAUTION: If amplification is not going to be done immediately, preserve the **DNA** at ≤-18ºC.

9.ENDOGENOUS CONTROL

Endogenous Control (EC) detection allows for evaluation of sampling, nucleic acid extraction and qPCR run and therefore identifies the presence of false negative results. Assay mix tube includes **a specific set of primers and probe for an endogenous control gene present in all animal cells. This target is detected through HEX or VIC channel and its Cq ranges between 22-37.**

When analyzing strains/microbiological isolations, the addition of and exogenous control is recommended.

Amplification of this EC does not interfere with detection of the **L. intracellularis** target **DNA** even when it is present at low copy number.

10.QUANTITATIVE AND QUALITATIVE PCR.

The kit can be used for quantitative or qualitative Real Time PCR. A specific **L. intracellularis** positive control with a determined number of copies (see CoA) is supplied with the kit.

To perform quantitative Real Time PCR, a standard curve must be prepared. To prepare the standard curve, make 6 ten-fold dilutions (10^{-1} to 10^{-6}) of the reconstituted PC in nuclease free water. (As an example, by diluting 50 µl of PC in 450 µl of nuclease free water). Vortex thoroughly and change the pipette tip between each serial dilution.

For the standard curve analysis, pipette 5 µl of each dilution into each well, according to your protocol plate set up.

Perform 2-3 replicates of each dilution to get more reliable quantification data. For quantification do not use a PC frozen-thawed over 3 times. Standard curve is not needed to perform qualitative Real Time PCR.

11.AMPLIFICATION OF THE GENETIC MATERIAL

1. Thaw the assay mix tube you need regarding the number of required reactions. Non used assay mix tube must remain frozen.

2. Vortex the tube briefly.

3. Dispense 15 µl of assay mix tube into each well/tube used for the assay.

4. Add 5 µl of nucleic acid sample or control (PC or negative control (water), or negative extraction control) in the corresponding wells/tubes used for the assay.

5. Seal the wells/tubes with their corresponding film or caps.

6. Centrifuge the plates/tubes gently before inserting it into the thermocycler to prevent drops in the well pit walls.

THERMAL PROFILE	Temperature (ºC)	Time	Cycles
Pre-PCR (RT step) [4]	45	15 min	1
Denaturation	95	5 min	1
Amplification	95	15 sec	42
	60[5]	60 sec	

[4] The RT step needs to be included in those assays targeting RNA pathogens. For DNA targets this step is not needed and can be omitted.

Those INgene PCR and RT-qPCR kits **that are provided in a ready to use format, share the same PCR thermal profile.** Therefore, they can be run together using the RT-qPCR protocol.

[5]Fluorescence data should be collected during this step through FAM (**L. intracellularis**) and HEX/VIC (EC) channels.

Unselect ROX as passive reference in those equipment that have this channel selected by default.

12.ANALYSIS AND INTERPRETATION OF RESULTS

SAMPLE (FAM) **Positive if Cq ≤ 38**	**POSITIVE CONTROL (FAM)**	**NEGATIVE CONTROL (FAM)**	**SAMPLE Endogenous control (HEX)**	**ASSAY RESULT**
POSITIVE	POSITIVE	NEGATIVE	DO NOT CONSIDER [6]	**VALID**
NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	**VALID**
NEGATIVE	POSITIVE	NEGATIVE	**NEGATIVE** [7]	NOT VALID
POSITIVE/NEGATIVE	**NEGATIVE**	NEGATIVE	POSITIVE/NEGATIVE	NOT VALID
POSITIVE/NEGATIVE	POSITIVE	**POSITIVE**	POSITIVE/NEGATIVE	NOT VALID

[6] Do not consider EC signal in Positive samples or Positive control.

[7] Evaluate purity and concentration of the sample. If needed, prepare ten-fold dilutions (10^{-1} to 10^{-2}) of this sample and re-test in the qPCR assay.
In case of persisting negative results in EC, a new nucleic acid extraction is necessary.

13.TECHNICAL ASSISTANCE AND ORDERS

For further information concerning the assay and its performance, please contact:

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