

## INgene q Paratuberculosis (multiplex)

R.10.XPT.K.5/50

50 Reactions

R.10.XPT.K.5/100

100 Reactions

qPCR kit for the detection of *Mycobacterium avium* subsp. *paratuberculosis* in biological samples. *Real Time PCR (TaqMan technology)*.



### 1. INTRODUCTION AND INTENDED USE

INgene q Paratuberculosis (multiplex) is a real-time PCR kit suited for the detection and/or quantification of *Mycobacterium avium* subsp. *paratuberculosis* (PTBC F57 and PTBC IS900) DNA in biological samples of clinical interest from Cattle, Small ruminants in a simple way, with high sensitivity and specificity.

The assay is suitable for the following sample types: faeces, rectal swabs, milk, tissues including intestine, ileocecal valve, lymph nodes and strains/vaccines.

*For research use only*

### 2. PRINCIPLE OF THE ASSAY

INgene q Paratuberculosis (multiplex) is an assay for amplification and detection of the PTBC F57 and PTBC IS900 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 PTBC F57 and PTBC IS900 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 PTBC F57 and PTBC IS900, 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 F57 gene at 530 nm, specific primers and a probe labelled with HEX/VIC fluorochrome for the detection of Endogenous control and CY5 fluorochrome for the detection of IS9 gene.

The kit includes a PTBC F57 and PTBC IS900 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 Paratuberculosis (multiplex) ASSAY MIX TUBE	Buffers, enzyme, dNTPs, primers/probes for the respective targets	1 or 2 <sup>1</sup>	750 µl
PC75 <sup>2</sup> POSITIVE CONTROL	Lyophilized specific synthetic control	1	Lyophilized
Water	Nuclease free water	1	1 ml

<sup>1</sup> 1 vial for 50 reactions format and 2 for 100 reactions format.

<sup>2</sup> 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  $\leq -18^{\circ}\text{C}$ .

#### 4. CONSERVATION OF THE KIT COMPONENTS

All components have to be stored at  $\leq -18^{\circ}\text{C}$  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  $\mu\text{l}$ ) 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.)
- DNeasy Blood & Tissue Kit (QIAGEN, Inc.)
- QIAamp Viral RNA Mini 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 respective 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  $\leq -18^{\circ}\text{C}$ .

**9. ENDOGENOUS CONTROL**

*Only consider this point if the kit contains Endogenous Control detection.*

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 a gene of E. coli strains (naturally present in faeces and digestive samples). This target is detected through HEX or VIC channel and its Cq ranges between 20-37.**

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

Amplification of this EC does not interfere with detection of the PTBC F57 and PTBC IS900 target DNA even when it is present at low copy number.

**10. 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  $\mu\text{l}$  of assay mix tube into each well/tube used for the assay.
4. Add 5  $\mu\text{l}$  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 ( $^{\circ}\text{C}$ )	Time	Cycles
Pre-PCR (RT step) <sup>3</sup>	45	15 min	1
Denaturation	95	5 min	1
Amplification	95	15 sec	42
	60 <sup>4</sup>	60 sec	

<sup>3</sup> 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.

<sup>4</sup> Fluorescence data should be collected during this step through FAM (F57 gene), HEX/VIC (Endogenous control) and CY5 fluorochrome for the detection of IS9 gene.

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

## 11. ANALYSIS AND INTERPRETATION OF RESULTS

SAMPLE Positive if Cq ≤ 38	POSITIVE CONTROL	NEGATIVE CONTROL	SAMPLE Endogenous control <sup>5</sup>	ASSAY RESULT
POSITIVE	POSITIVE	NEGATIVE	DO NOT CONSIDER <sup>6</sup>	VALID
NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	VALID
NEGATIVE	POSITIVE	NEGATIVE	<b>NEGATIVE</b> <sup>7</sup>	NOT VALID
POSITIVE/NEGATIVE	<b>NEGATIVE</b>	NEGATIVE	POSITIVE/NEGATIVE	NOT VALID
POSITIVE/NEGATIVE	POSITIVE	<b>POSITIVE</b>	POSITIVE/NEGATIVE	NOT VALID

<sup>5</sup> Only consider this column if the kit contains EC detection.

<sup>6</sup> Do not consider EC signal in Positive samples or Positive control.

<sup>7</sup> 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.

**How to interpret the results:**

IS900 pos and F57 pos                      confirmed as MAP

IS900 pos (Cq < 33) and F57 neg              confirmed as not MAP

IS900 pos (Cq > 33) and F57 neg              not confirmed as MAP

## 12. TECHNICAL ASSISTANCE AND ORDERS

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

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