

MAP qPCR kit

Cat: LTQPCR-MAP

Quantity 50 tests

1. Purpose

Mycobacterium avium paratuberculosis (MAP) is an obligate intracellular bacterium that has frequently been associated with Johne's disease (JD), a non-treatable chronic granulomatous enteritis of cattle and small ruminants. JD is associated with profuse diarrhea, emaciation, submandibular edema, and eventually death of infected animals due to poor nutrient absorption.

The «MAP qPCR kit» is intended to detect MAP DNA in tissues, swabs, milk, feces and cultural material, which is rapid, accurate and easy-to-operate.

The test kit «MAP qPCR kit» is designed for multiplexed detection of the specific to MAP DNA (IS900 gene), according OIE validated protocol, and Internal Control (Ruminant) gene as sample processing control by real time polymerase chain reaction (PCR) with fluorescent detection of the analysis results.

2. Clinical significance

JD is prevalent worldwide and imposes considerable economic burden to the cattle industry due to production losses. Clinical manifestation of JD appears too late, more than a year after infection, making difficult an early diagnosis of the disease, thus, only PCR can provide early diagnosis and eradication of infection from the herd.

3. Principle of analysis

The method is based on the isolation of total DNA from samples, and further amplification of specific regions of MAP DNA (IS900 gene) using specific oligonucleotide primers and the synthesis of complementary DNA chains using the enzyme Hot start Taq-DNA polymerase. Fluorescent detection of the analysis results is performed both in real-time (Real-time mode) or end point assay using two fluorescence detection channels (FAM525 nm, ROX580 nm), table 1.

Table 1. Target specificity

Marker	Detection channel
MAP, IS900 gene	FAM
Internal control, Ruminant gene	ROX

4. Kit contents:

Table 2. PCR reagents:

Component name	Quantity	Volume
MAP Master Mix (Lyophilized)	1 vial	
MAP Positive Control (Lyophilized)	1 tube	

5. Storage and stability

The test kit reagents are stable for the shelf life indicated on the label (at least 12 months from manufacture date) if stored at room temperature.

6. Sample preparation

Tissues, swabs, feces and cultural material samples can be processed according commercially available DNA extraction kit recommendations. We recommend using our Nucleic acids extraction kit compatible with automatic Nucleic acid extraction device LTEXT1.

7. Reagent preparation

Master Mix preparation: Add distilled water 0,5 mL directly to Lyophilized Master Mix, wait 5 min, mixing well and dispense 10 µL into PCR testing tubes.

Positive control preparation: Add distilled water 0,5 mL directly to Lyophilized Positive Control, wait 5 min, mixing well and use directly 10 µL for PCR or use 100 µL for Nucleic acid extraction (to control Nucleic acid extraction efficiency). Reagents and controls must be kept in a chilled stand. After use reagents and controls can be freeze at minus 20°C for storage. Several freezing-thawing cycles are allowed.

8. Assay procedure

8.1. Select the required amount of the PCR tubes or wells in the PCR plate.

8.2. Using pipette tips with an aerosol filter dispense Master mix 10 µL add 10 µL of DNA samples (Positive and Negative control samples).

8.3. Place testing tubes in the fluorescence PCR instrument, and set the negative/positive control and unknown samples parameters for PCR reaction according to the instrument operating instructions. Fluorescence is measured at 60°C on the FAM, and ROX channels. The PCR parameters are shown in the table 3:

Table 3. PCR run method parameters

Step	Temperature	Time	Repeats	Data collections
1	95 C	5 min	1	No
2	95 C	10 sec	45	No
	60 C	30 sec		Yes

8.6. Start run.

9. Result records and their interpretation is shown in the table 4:

Table 4. PCR results interpretation

Marker	Fluorescent channels	MAP Positive	MAP Negative
MAP IS900 gene	FAM	Ct ≤ 45	Undetectable
Internal control Ruminant gene	ROX	Ct ≤ 35	Ct ≤ 35