Product Testing Report

G240. Mycoplasma qPCR Detection Kit

Applied Biological Materials Inc.
3671 Viking Way, Unit 1
Richmond, BC, Canada
V6V 2J5
www.abmgood.com

Product Efficiency and Sensitivity

Purpose

To evaluate the amplification efficiency, sensitivity, and specificity of the Mycoplasma qPCR Detection Kit using a tenfold serial dilution of positive control DNA ranging from 10^9 to 1 copy.

Method

A tenfold serial dilution of the provided positive control DNA was prepared, ranging from 10^9 copies down to 1 copy per reaction. Each dilution was run in duplicates, following the standard assay protocol. Each 20 µl qPCR reaction contained 10 µl of MegaFi™ Pro 2X qPCR MasterMix, 1 µl Primer Mix, 2 µl of diluted template, and nuclease-free water to volume. A No Template Control (NTC) was included to assess specificity. Thermal cycling was performed with initial enzyme activation at 95 °C for 10 minutes, followed by 40 cycles of 95 °C for 15 seconds and 63 °C for 45 seconds. A melting curve was generated post-amplification.

To confirm amplicon size and reaction specificity, PCR products were analyzed on a 2% agarose gel, with the expected 270 bp band used as the reference.

Following data acquisition, Ct values were plotted against the $\log_{10}$ of template copy number to construct a standard curve. Amplification efficiency was calculated from the slope of the curve using the formula:

$$E = -1 + 10^{\left(-\frac{1}{slope}\right)}$$

Figure 1 – Mycoplasma qPCR Detection Kit demonstrates a highly linear qPCR standard curve from a tenfold serial dilution of positive control DNA (10^9 to 1 copy).

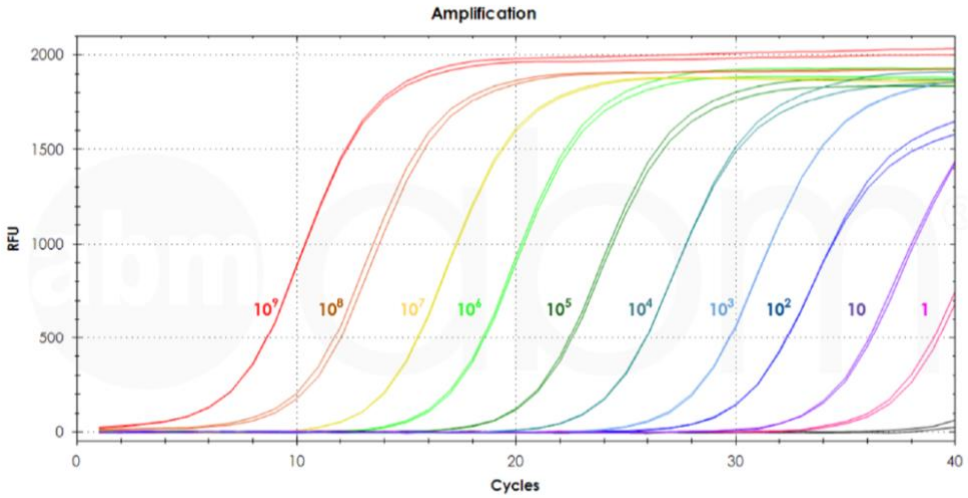


Figure 2 – Mycoplasma qPCR Detection Kit enables robust low-copy detection with high specificity across a tenfold serial dilution of positive control DNA (10^9 to 1 copy).

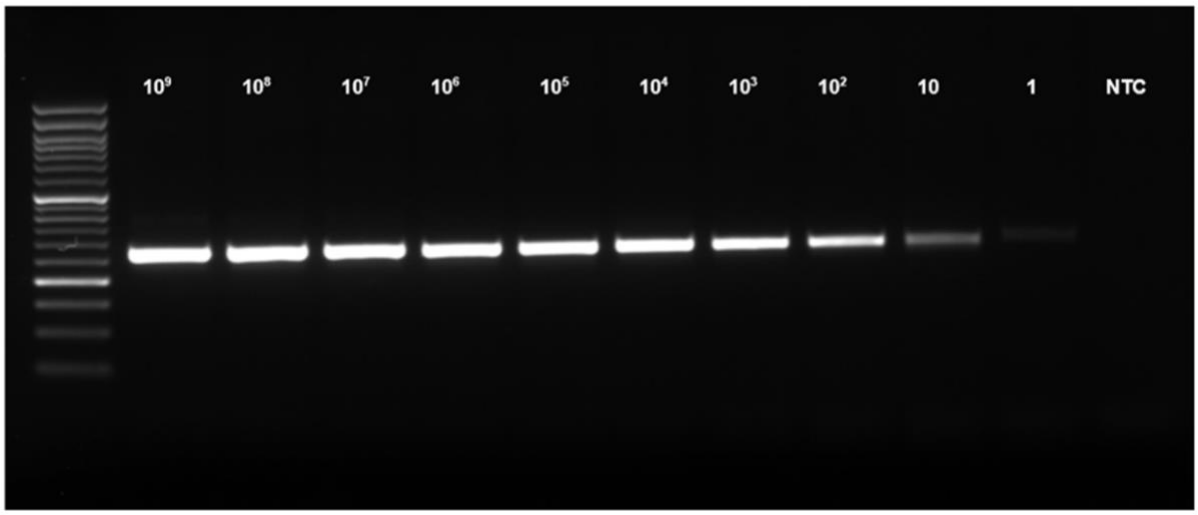
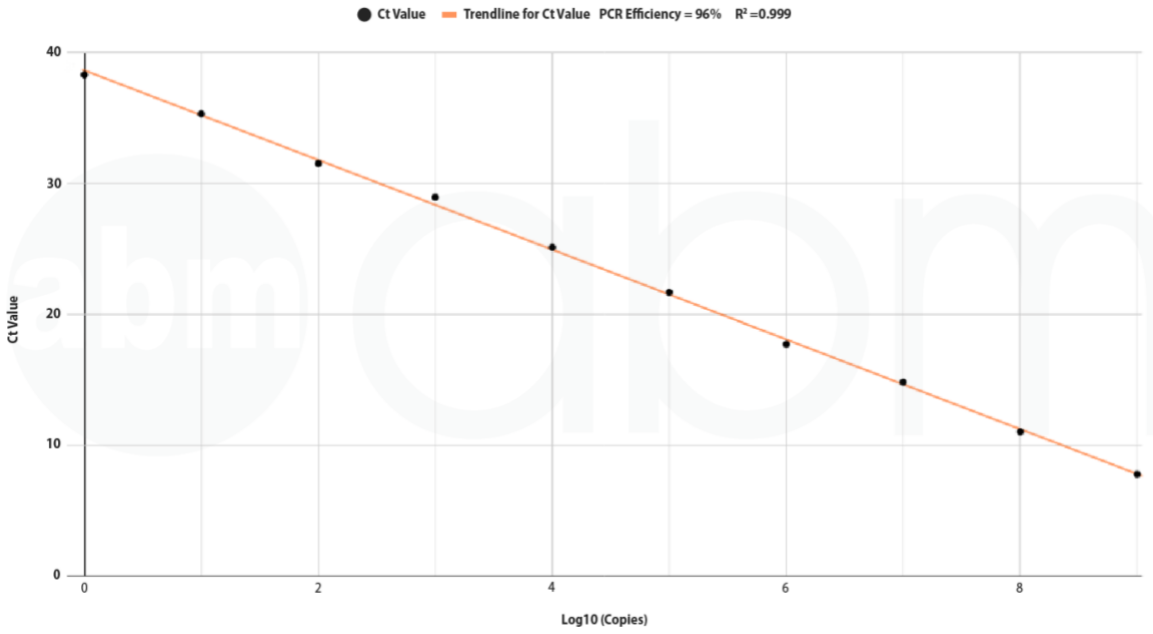


Figure 3 – Mycoplasma qPCR Detection Kit shows a qPCR standard curve with high amplification efficiency (96%).



Result

The qPCR amplification plot demonstrated a highly linear response across the full dilution range. Clear, well-separated curves were observed from 10^9 to 1 copy, with earlier Ct values at higher template inputs, indicating high assay sensitivity. No amplification was seen in the NTC, confirming assay specificity.

A standard curve generated from the Ct data showed excellent linearity (R^2 = 0.999) and a calculated PCR efficiency of 96%, supporting robust performance across a broad dynamic range.

Agarose gel analysis confirmed the presence of a distinct 270 bp band at every dilution point. Band intensity decreased proportionally to input DNA, and no non-specific bands or primer-dimer artifacts were observed, including at low-copy levels. The NTC lane was negative, consistent with the qPCR data.

Conclusion

Mycoplasma qPCR Detection Kit exhibits excellent efficiency (96%), sensitivity down to a single copy, and high specificity with no background amplification. These features make it a robust and reliable tool for the sensitive detection of mycoplasma contamination in molecular workflows.

Mycoplasma Detection Sensitivity and Specificity

Purpose

To validate the specificity and diagnostic accuracy of the Mycoplasma qPCR Detection Kit using control DNA and a test sample known to be positive for mycoplasma contamination.

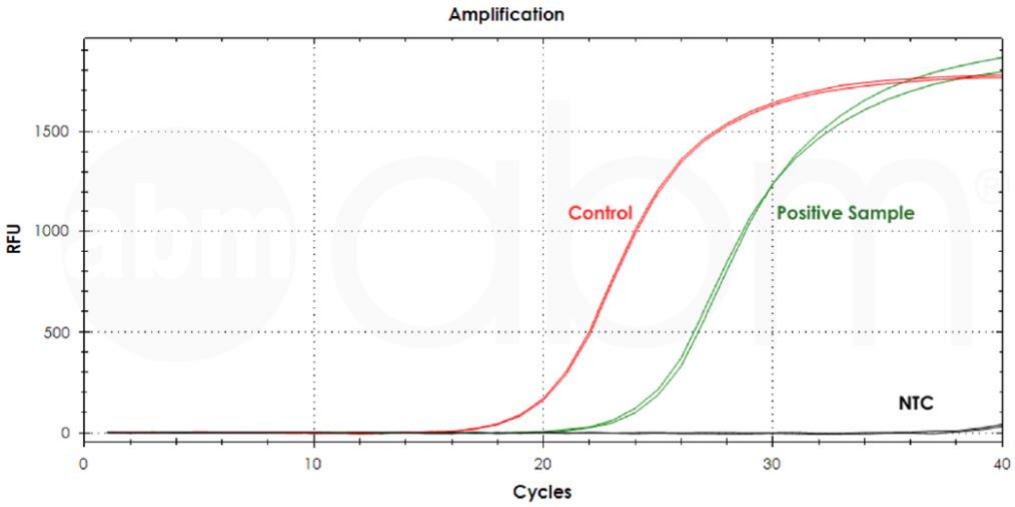
Method

To prepare the sample, 5 µl of culture media was collected from a confluent cell culture and diluted with 20 µl of nuclease-free water (1/5 dilution) to reduce potential PCR inhibitors.

Each 20 µl qPCR reaction contained 10 µl of MegaFi™ Pro 2X qPCR MasterMix, 1 µl Primer Mix, 2 µl of the diluted test sample or Positive Control, and nuclease-free water to volume. Reactions were run in duplicate, with a No Template Control (NTC) included to confirm specificity.

Thermal cycling was performed with an initial activation at 95 °C for 10 minutes, followed by 40 cycles of 95 °C for 15 seconds and 63 °C for 45 seconds. A melting curve was included after amplification.

Figure 4 - Mycoplasma qPCR Detection Kit accurately detects mycoplasma contamination.



Result

Amplification curves showed clear signals for both the control DNA (Red) and the test sample (Green), confirming mycoplasma contamination in the culture. Ct values for the sample were within the expected detection range. The NTC showed no amplification, demonstrating high assay specificity.

Conclusion

Mycoplasma qPCR Detection Kit delivers reliable and sensitive detection of mycoplasma contamination in cell cultures. The absence of amplification in the NTC further confirms the assay's specificity and robustness for routine screening.

Cross-Species Detection of Mycoplasma and Acholeplasma

Purpose

To evaluate the performance of the Mycoplasma qPCR Detection Kit across a broad range of clinically and experimentally relevant mycoplasma and acholeplasma species.

Method

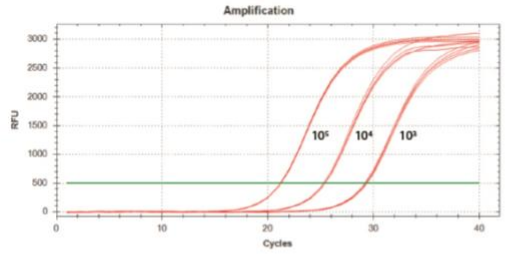
Plasmids containing the 16S rRNA gene from seven microbial species—*M. pneumoniae, M. arginini, M. orale, M. hyorhinis, M. hominis, M. fermentans,* and *Acholeplasma laidlawii*—were serially diluted to generate three template input levels: 10^5, 10^4, and 10^3 copies per reaction. Each dilution was tested in quadruplicate following the standard assay protocol.

Each 20 µl qPCR reaction contained 10 µl of MegaFi™ Pro 2X qPCR MasterMix, 1 µl of species-specific Primer Mix, 2 µl of diluted plasmid DNA, and nuclease-free water to volume. All reactions were assembled on ice, and a No Template Control (NTC) was included for each species to assess specificity.

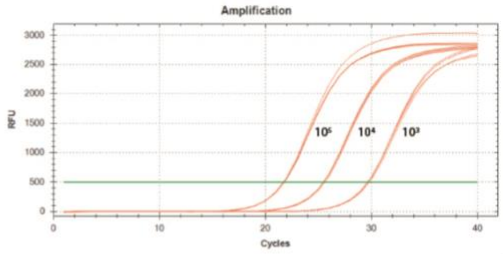
Thermal cycling was carried out with an initial enzyme activation at 95 °C for 10 minutes, followed by 40 cycles of 95 °C for 15 seconds and 63 °C for 45 seconds. Fluorescence signals were collected during the extension step, and Ct values were recorded for all replicates.

Figure 5 - Broad-Spectrum detection of Mycoplasma and Acholeplasma species using the Mycoplasma qPCR Detection Kit.

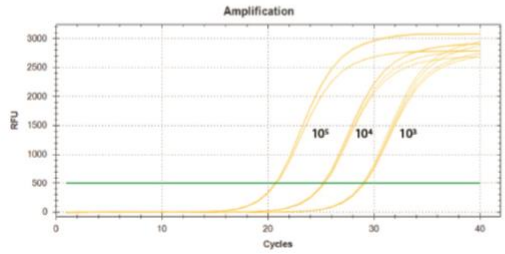
M. pneumoniae



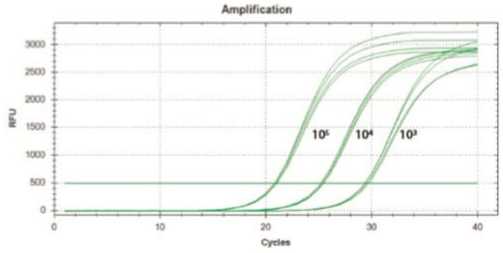
M. arginini



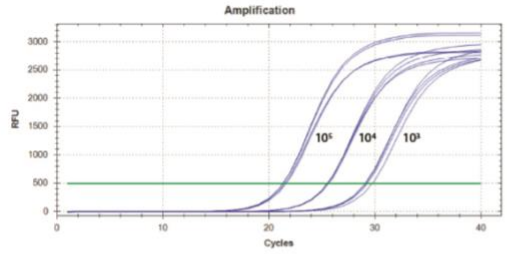
M. orale



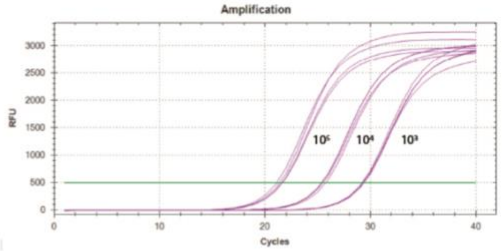
M. hyorhinis



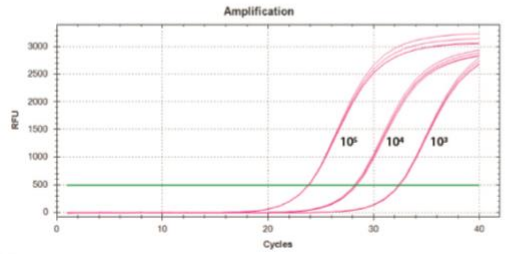
M. hominis



M. fermentans



A. laidlawii



Average CT values from 10^5 to 10^5 copies

Species	10^5 copies	10^4 copies	10^3 copies
M.pneumoniae	21.21	25.34	29.27
M. arginini	21.72	25.48	29.67
M. orale	21.70	25.24	29.03
M. hyorhinis	21.92	25.31	29.49
M. hominis	21.43	25.54	29.31
M. fermentans	21.41	25.47	29.28
A. laidlawii	23.81	28.28	32.41

Result

All seven species showed clear and consistent amplification curves across the full dilution range. The assay demonstrated high sensitivity, with reliable detection down to 10^3 copies. Amplification was efficient and reproducible, with average Ct values exhibiting tight clustering across replicates and species. For example, *M. pneumoniae* amplified with Ct values of 21.21 (10^5 copies), 25.34 (10^4), and 29.27 (10^3), while *A. laidlawii* showed Ct values of 23.81, 28.28, and 32.41, respectively.

Conclusion

Mycoplasma qPCR Detection Kit provides robust, sensitive, and reproducible detection across a broad range of mycoplasma and acholeplasma species. The assay's consistent Ct values and cross-species performance highlight its reliability for comprehensive contamination screening in research, bioproduction, and quality control workflows.