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Product Information

Venor™GeM Mycoplasma Detection Kit, PCR-based

Catalog Number **MP0025**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

The Venor™GeM Mycoplasma Detection Kit utilizes the polymerase chain reaction (PCR), which was established as the method of choice for highest sensitivity in the detection of *Mycoplasma* and *Acholeplasma* contamination in cell cultures and other cell culture derived biologicals. Detection requires as little as 1–5 fg of mycoplasma DNA corresponding to 2–5 mycoplasma per sample volume.

The primer set is specific to the highly conserved rRNA operon, or more specifically, the 16S rRNA coding region in the mycoplasma genome. This allows for detection of all *Mycoplasma*, *Acholeplasma*, and *Ureaplasma* species tested so far, which are usually encountered as contaminants in cell cultures. Eukaryotic and bacterial DNA are not amplified by this kit.

Only one protocol is needed for the detection of all mycoplasma species. The detection procedure can be performed within 3 hours. This kit also provides internal control DNA, which can be added to the reaction. When running the PCR with the internal control DNA, a successfully performed reaction is indicated by a distinct 191 bp band on the agarose gel.

Components

Primer set and nucleotides Lyophylized primers and deoxynucleotide triphosphates dATP, dCTP, dGTP and dTTP; aliquoted for 25 reactions	red cap P9242
PCR 10× reaction buffer sterile, 500 µl 100 mM Tris-HCl (pH 8.5) 750 mM KCl 30 mM $MgCl_2$	blue cap P9367
Positive Control DNA non-infectious DNA-fragments of *Mycoplasma orale* genome prepared by PCR, lyophilized	green cap P9117
Internal Control DNA Lyophilized plasmid DNA including mycoplasma-specific primer sequences and an internal sequence of the HTLV-I *tax* gene with a size of ~191 bp, non-infectious	yellow cap I0532

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. It is not for clinical diagnostics or testing of human samples. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Kit components are stable during shipping at ambient temperature. Upon receipt, store at 2–8 °C. After rehydration of the primer/nucleotide mix, the positive control, and the internal control, store below –20 °C and avoid repeated freezing and thawing. For repeated testing of low sample numbers, primer/nucleotide mix and controls should be aliquoted after rehydration. By following these recommendations, the kit is stable until the expiration date stated on the label.

Procedure

Preparation of Sample Material

Cell lines should be pre-cultured in the absence of antibiotics for several days to maximize test sensitivity. Samples should be derived from cultures that are at 90–100% confluence. PCR inhibiting substances may accumulate in the medium of older cultures. For these sample materials a DNA extraction is strictly recommended prior to testing.

To avoid false positive results, it is recommended to use deionized, DNA-free water, aerosol-preventive filter tips, and gloves.

Templates for PCR analysis are prepared by boiling the supernatant of cell cultures or other biologicals for 5 minutes as follows:

1. Transfer 100 µl of supernatant from the test culture to a sterile microcentrifuge tube (Catalog Number T0447). The lid should be tightly sealed to prevent opening during heating.

2. Heat the sample supernatant at 95 °C for 5 minutes.

3. Briefly centrifuge (5 seconds) the sample supernatant to pellet cellular debris before adding to the PCR mixture.

Rehydration of the Reagents

1. Before rehydrating the tubes, centrifuge the tubes to ensure that the lyophilized components are spun down (5 seconds at maximum speed).

2. Add the appropriate amount of deionized, DNA-free water (Catalog Number W1754):

primer/nucleotide mix (per portion of 25 reactions)	65 µl
positive control	300 µl
internal control	300 µl

3. Incubate for 5 minutes at room temperature.

4. Vortex and centrifuge again.

5. Keep reagents on ice and store below –20 °C after rehydration.

Thermal Profile

1. The programming process of your cycler is explained in the instrument's manual.
2. The incubation time depends on the polymerase used. Hot start enzymes need to be activated at 94 °C. Please see polymerase data sheet for duration.

Thermal Cycle Program

1 cycle	94 °C for 2 minutes
39 cycles	94 °C for 30 seconds
	55 °C for 1 minute
	72 °C for 30 seconds
	cool down to 4–8 °C

The PCR Mastermix

Total volume per reaction is 25 µl. When setting up reactions, calculations should also include positive and negative controls.

1. Preparation of a PCR Mastermix for use with Jumpstart™ *Taq* polymerase (Catalog Number D9307), with a final $MgCl_2$ concentration of 3.0 mM, is detailed in Table 1. The mastermix can also be prepared for 25 reactions, aliquoted as needed, and stored below –20 °C for up to 3 months. Aliquot 23 µl of PCR mastermix into each PCR reaction tube. For other polymerase concentrations the amount of water needs to be adjusted.

Table 1.
Pipetting schemes for Preparation of PCR Mastermix

	1 reaction	**3 reactions**	**25 reactions***
Water	15.1 µl	45.3 µl	377.5 µl
10× reaction buffer (blue cap)	2.5 µl	7.5 µl	62.5 µl
Primer/ nucleotide mix (red cap)	2.5 µl	7.5 µl	62.5 µl
Internal control (yellow cap)	2.5 µl	7.5 µl	62.5 µl
Taq Polymerase (2.5 U/µl) Catalog Number D9307	0.4 µl	1.2 µl	10 µl

* 25 reactions are the equivalent of the content of one red-capped vial.

Note: Use of other polymerases is possible; however, the PCR buffer supplied with the enzyme should be used. The final $MgCl_2$ concentration must be adjusted to 3.0 mM. For PCR buffers with separate $MgCl_2$ solutions, Table 2 may be used a guide for the volume of the $MgCl_2$ solution used in a 25 µl PCR reaction.

Table 2.
Volume of Separate $MgCl_2$ Solutions to Prepare Other PCR Buffers

$MgCl_2$ Solution Concentration	Volume of $MgCl_2$ Solution per PCR reaction
25 mM	3.0 µl
50 mM	1.5 µl
100 mM	0.75 µl

2. Add 2 µl of deionized, DNA-free water as a negative control into reactions tubes and seal to avoid contamination.

3. Add 2 µl of sample (as previously described) to PCR reaction tube per sample being tested and seal.

4. Pipette 2 µl of positive control DNA into positive control reaction tube.

5. Proceed to thermal cycling

Agarose Gel Run

1. Use 1.5 % standard agarose gel (Catalog Number A9539) with 5 mm comb.

2. Load 5 µl of each PCR reaction, mixed with bromophenol blue loading buffer (Catalog Number G7654) per lane.

3. Stop electrophoresis after 2 cm run distance (depending on the electrophoresis chamber used e.g., run for 20 minutes at 100 V).

Results

Gel Evaluation

1. If internal control DNA was used, a distinct 191 bp band should appear in every lane indicating a successfully performed PCR. This band may fade out with increased amount of amplicons formed, caused by mycoplasma DNA loads of $>5 \times 10^6$ copies/ml.

2. No amplification of control DNA may be due to the following reasons: (1) activity of *Taq* polymerase is insufficient; (2) reaction buffer is not suitable for polymerase; (3) control DNA tubes have not been spun down before rehydration; (4) programming mistake; and (5) pipetting mistake.

3. Before rerun of a negative and a positive control please check thermocycler protocol and pipetting scheme. Polymerase concentration can by raised up to 2.5 U/reaction. The VenorGeM buffer can be replaced by the specific buffer provided with the polymerase; however, the magnesium concentration must then be adjusted to 3.0 mM.

4. The initial concentration of positive control DNA exceeds 5×10^6 copies/ml in order to account for DNA loss resulting from repeated freeze/thaw cycles.

5. With VenorGeM designed for high sensitivity and therefore, prone to nonspecific annealing, bands of various lengths that are less intensive can be produced, but do not indicate positive results. Possible primer self-annealing produces another band of 80–90 bp in length, but also does not affect the precision or results of the test.

6. If the PCR of a sample is inhibited, PCR inhibitors can easily be removed from the sample by performing a DNA extraction with a commercially available kit. (Catalog Numbers G1N10, G1N70, or NA2000).

Table 2.
Relevant amplicon sizes

Internal control	191 bp
Mycoplasma sp. (see Appendix)	~270 bp

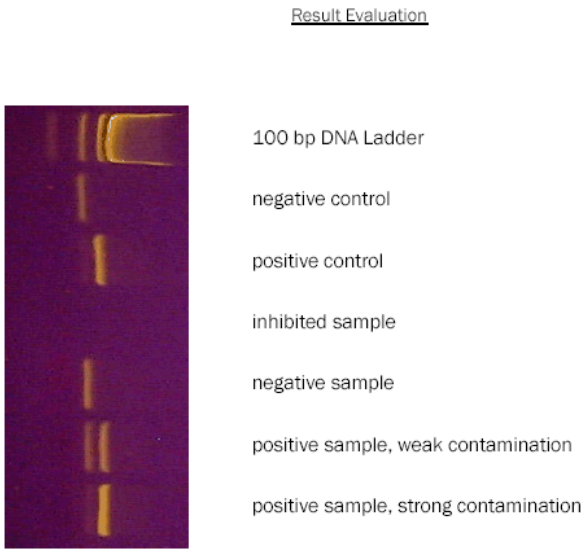
Table 3.
Results of successfully performed PCR

PCR sample	**Band pattern**
Negative control	Band at 191 bp
Positive control	Band at 267 bp, possibly an additional band at 191 bp

Table 4.
Interpretation of possible band patterns

Band pattern	**Interpretation**
Band at 191 bp	Negative sample
Bands at 270 bp and 191 bp	Mycoplasma-positive sample with weak contamination
Strong band at 270 bp	Mycoplasma-positive sample with strong contamination
No band	PCR inhibition, insufficient polymerase activity

Figure 1.



Appendix

Detection Range and Sizes of Amplicons

No.	species	amplicon size (bp)
1	*Mycoplasma pneumoniae*[2]	277
2	*Mycoplasma penetrans*	274
3	*Mycoplasma pirum*	274
4	*Acholeplasma laidlawii*[2]	273
5	*Mycoplasma fermentans*	272
6	*Ureaplasma urealyticum*	271
7	*Mycoplasma hyorhinis*[2]	269
8	*Mycoplasma pulmonis*	268
9	*Mycoplasma falconis*	268
10	*Mycoplasma orale* [1,2]	267
11	*Mycoplasma arthritidis*	267
12	*Mycoplasma arginini*	267
13	*Mycoplasma spermatophilum*	267
14	*Mycoplasma opalescens*	267
15	*Mycoplasma primatum*	267
16	*Mycoplasma maculosum*	267
17	*Mycoplasma bovis*	267
18	*Mycoplasma cloacale*	266
19	*Mycoplasma hyosynoviae*	266
20	*Mycoplasma synoviae*[2]	266
21	*Mycoplasma salivarium*	266
22	*Mycoplasma faucium*	265
23	*Mycoplasma hominis*	267
24	*Mycoplasma genitalium*	267

[1] provided as positive control DNA
[2] Test strain according to European Pharmacopoeia, Suppl. 2000, 2.6.7. Mycoplasmas