Troubleshooting and FAQ

Detection of Mycoplasma with Venor®GeM.

Please find detailed anwers on Frequently Asked Questions concerning Venor®GeM detection of mycoplasma below.

Cambio Ltd, 1 The Irwin Centre, Scotland Road, Dry Drayton, Cambridge, CB23 8AR, UK
Tel :+44 (0)1954 210 200 , +44 (0)1954 210 300, email: support@ cambio.co.uk
www.cambio.co.uk

Can this system be used for cells in culture, primary cells, virus stocks and other biologicals?

The Venor®GeMSystem is designed for the detection of mycoplasmas in cell cultures and virus stocks as well as many other biological samples. Cell samples may be derived from either cultured cells, tissue extracts, or directly from cryostocks.

What sample material can be tested with Venor®GeM?

The prescribed mycoplasma detection protocol employs the testing of the supernatant. However, other materials that can be tested are Fetal Calf Serum, vaccines, and paraffin-embedded samples following DNA extraction.

What sample size is required?

The Venor®GeM System requires as little as 2 µl sample volumen for one reaction. The cells should be cultivated to 90% confluence to guarantee a maximal density of mycoplasma in the sample volume.

How sensitive is Venor®GeM?

Venor®GeM is the most sensitive PCR Mycoplasma Detection Kit available on the market. Detection can be accomplished with as little as 1-5 fg of mycoplasma DNA that corresponds to 2-5 mycoplasma per sample volume.

What is the detection range of Venor®GeM ?

Venor®GeM tests at least for 26 different mycoplasma species. A complete list of these species is available within this Instruction Manual. For further information pertaining to the detection range of Venor®GeM, a downloadable version (pdf format) of the Interlaboratory Validation Study is available on our website.

Is the intensity of the PCR amplicon proportional to the contamination level?

Venor®GeM for conventional PCR is a qualitative test for mycoplasma contamination. A band at between 265-278 bp depicts a positive result regardless of its intensity. A band of weak intensity represents a low

contamination level, whereas a band of greater intensity represents a high contamination level. For quantification of the mycoplasma contamination level you should use our Venor®GeM for RealTime PCR.

The test has resulted in non-specific bands.

A PCR amplicon at a non-specific band (i.e. 300 bp) represents a positive result when this non-specific band corresponds in length to the amplicon of the positive control. If these non-specific bands do not correspond to the positive control, they then represent a negative result. Venor®GeM is highly sensitive, and therefore prone to non-specific annealing which may generate less intensive bands of various lengths. However, such bands 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.

Can the processed samples be stored for later analysis?

After heat treating the cell culture supernatant (500 µ l, 10 min, 95°C), samples can be stored for several days at 4°C prior to analysis. For long-term storage, it is recommended that samples be stored at -20°C either in their native state or after heat inactivated.

What are the storage conditions for the PCR products?

The PCR products are stable for one to two days at room temperature. For longer storage, the PCR products should be kept at -20°C, at which temperature they can be kept stable for longer than one year.

Is UV irradiated water necessary for the rehydration of the PCR primer set, nucleotides, and controls?

The water used for rehydration must be free of DNA. We recommend UV-irradiated water, however freshly distilled water, gamma-irradiated water, or any other method for obtaining DNA-free water is also acceptable.

What controls should be performed?

It is highly recommended that both positive and negative control reactions be performed for each test series. These controls are to insure assay conditions, as well as biological positive controls. A negative control reaction, in which the sample volume is replaced by sterile water, should also be performed.
Is an internal control necessary?
The internal control is necessary to ensure the quality of the PCR. Samples should be derived from cultures which are at 80-90% confluence. PCR inhibiting

substances may accumulate in the medium of older cultures. The internal control band at 191 bp ensures a successfully performed reaction with no inhibition.

Does an internal control reduce sensitivity?

No, the internal control does not reduce sensitivity. The oligonucleotide primer set and nucleotides are designed at optimized concentrations.

What type of DNA polymerase can be used?

We highly recommend our reliable hot-start MB Taq DNA Polymerase (Cat. No. 53-0200) for optimal Venor®GeM performance and most sensitive results. We cannot guarantee excellent results with other polymerases.

Can a separate buffer, other than the universal buffer supplied with the kit, be used?

It is possible to substitute the Venor®GeM PCR 10x reaction buffer with the specific buffer supplied with the Taq DNA polymerase. However, the magnesium concentration of the buffer must then be adjusted to 3.0 mM. ann separat über Minerva Biolabs bezogen werden (Art.-Nr. 53-0200).

Do I need fixative agents for sample preparation?

Venor®GeM eliminates the use of hazardous chemicals found in most standard fixatives used in cell culture technique (e.g., formaldehyde) that also interfere with the polymerase reaction. Samples are prepared and stored after a simple heat inactivation of five minutes at 95°C, which is sufficient for mycoplasma DNA extraction and gives the best results in testing.

What kind of inhibitory substances in the media of old cultures interfere with the PCR reaction?

Which inhibitory substances in the media of old cultures exactly interfere with the PCR reaction is not known. In general, these are metabolites since fresh sera or media does not interfere with the PCR. Strikingly, 37% of all samples sent to Minerva Biolabs GmbH for testing mycoplasma contain inhibitory substances. (Note: If the media is yellow, you will certainly face inhibition of your PCR reaction)

What cell density in respect to suspension cell lines would be optimal for a PCR test? What is the general set-up you would recommend?

The supernatant of suspension cell lines (non-adherent) should be applied to test for mycoplasma. Cells should not be tested, since debris will interfere with the PCR reaction. With average titers at 10 6 and a maximum titer at 10 8 you will find sufficient mycoplasma in the supernatant to guarantee a sensitive PCR.

What differences exist between the sample kit and the regular kit?

The only differences are the volumes for resuspension and the mastermix-composition.
The sample kit can be used for 5 reactions, whereas the regular kit is commercially available for 25, 50, 100 and 250 reactions.

Does Mycoplasma penetrans resides intracellularly?

There is no definite answer to this question, since the appearance of intracellular *M. penetrans* still needs to be demonstrated.

The positive control band is not visible.

The positive control DNA is a "non-sticky" substance and thus may not remain at the bottom of the tube. Before rehydration of the positive control DNA (as well as the internal control DNA and the primer nucleotides), the tubes must be briefly centrifuged before opening. This will ensure that the lyophilized pellet is brought to the bottom of the tube, and thus will not be lost when opening.

What is the concentration of the positive control provided with Venor®GeM ?

The positive control contains amplicon DNA of a small fragment size. This can not be diluted properly to give reliable results. The positive control is only for better reading of positive results. The concentration of the positive control can vary slightly from lot to lot. Additionally, the material is not in the right conditions to allow a good quantification according to the high sensitivity of the test system. As our products are lyophilised for highest stability this condition is not optimal to recover exact amount of DNA from a tube wall for quantification purposes. Out of these reasons we offer special quantification standards from different microorganisms prepared especially for these applications.