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XpressMyco Master PCR Detection Kit

Catalog No.: XMYC2-25, XMYC2-50

Description

The XpressMyco Master PCR Detection Kit is designed for the routine examination of cell cultures for mycoplasmas. Extensive isolation of the genomic DNA is not necessary — a sample of the cell culture supernatant is used as template for the PCR. With few pipetting steps for the PCR approach and analysis by means of gel electrophoresis, the procedure delivers results quickly, easily, and to a high level of sensitivity.

Specifications

Application	PCR kit for routine screening of mycoplasmas in cell culture
Contents	• Lyophilized Master Mix: Hot-Start Taq-polymerase, primer mix, nucleotide triphosphates (dNTPs), buffer, MgCl2, stabilizers, loading buffer with tracking dye • Internal control: Plasmid with shortened 16S rRNA consensus sequence and corresponding primer binding sites • PCR grade water
Assays	25 or 50 applications per kit
Sensitivity	> 80 Mycoplasma genome copies
Shipping	Ambient Temperature
Storage	≤ 20°C

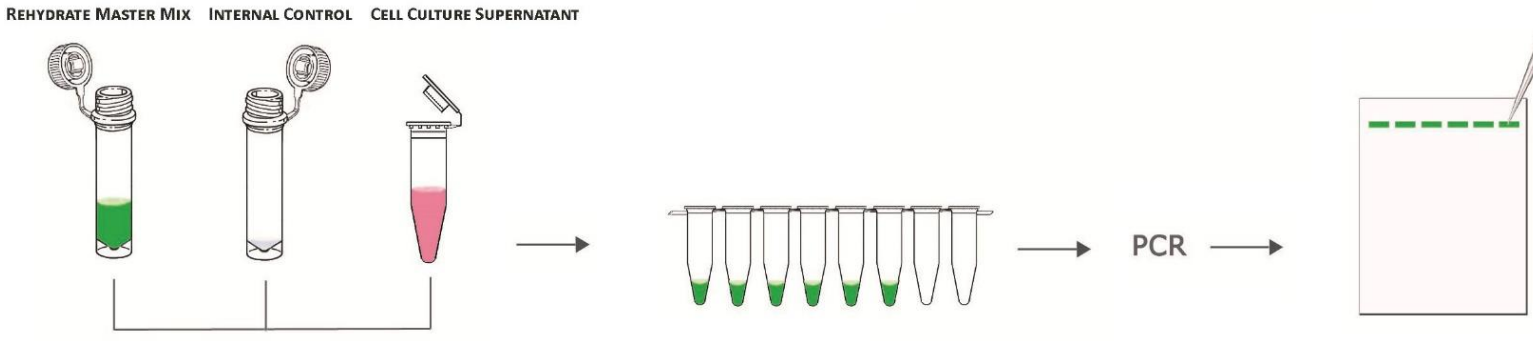
Important Information

These reagents are developed and sold for research purposes and in vitro use only. It is not intended for human or animal therapeutic or diagnostic purposes.

Contamination Precaution

To avoid false positive results, wear gloves while preparing the templates and the reaction mixtures for PCR. To avoid cross-contamination between samples, we recommend using aerosol-resistant pipet tips throughout the whole protocol. Furthermore, separate the area of sample preparation from the bench space in which the reaction mixtures for PCR are prepared.

Protocol



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Sample Preparation

1. Transfer 100 µl supernatant from the cell culture you wish to examine into a PCR tube.

   Note: At the time of harvesting the supernatant from the cell culture, cells should cover approximately 90% of the growth surface. The supernatant may cause PCR inhibition in excessively dense cell cultures.

2. Incubate the supernatant at 94°C for 5 minutes.
3. Spin the sample at 13,000 x g for 5 minutes to pelletize cell debris.
4. Use 2 µl of the supernatant as the template for the PCR.

Master Mix Preparation

Prior to first use, the lyophilized Master Mix must be rehydrated. For this purpose, pipette 575µl of water (included in the kit) to the lyophilizate and dissolve completely by inverting the tube several times. This amount is sufficient for 25 reactions. If not completely consumed, the rehydrated master mix must be stored immediately after use at ≤ -20° C. Multiple freezing and thawing does not affect reactivity.

PCR Preparation

For optimal reliability, we recommend performing any PCR approach in which the cell culture supernatant is tested with the internal control, even though this slightly reduces the sensitivity of detection. The internal control confirms the absence of PCR inhibitors and excludes false-negative results.

In addition, schedule a reaction without a template. This control reaction ensures that there are no contaminants of the reaction components with genetic material.

Transfer the following volumes to individual PCR tubes:

Note: Centrifuge all reagent tubes at low speed to ensure that the liquid is at the bottom of the tube.

Components	**Test sample with internal control**	**Control Reaction**
Master Mix	22.0 µl	22.0 µl
Internal Control	1.0 µl	1.0 µl
Test Sample	2.0 µl	–
Water	–	2.0 µl
Final volume	25.0 µl	25.0 µl

PCR Program

The following program yields optimal amplification of the internal control and genome copies from different mycoplasma species.

Temperature(°C)	**Time (s)**	**Function**	**Number of cycles**
94	60	Initial denaturation and activation of Taq	1 cycle
94	30	Denaturation	35 cycles
62	30	Annealing	
72	60	Polymerization	
72	180	Final elongation	1 cycle
4	∞		

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Expected products:

~200 bp Internal Control

~500 bp amplicon of the Mycoplasma genome

Electrophoresis of the PCR Products

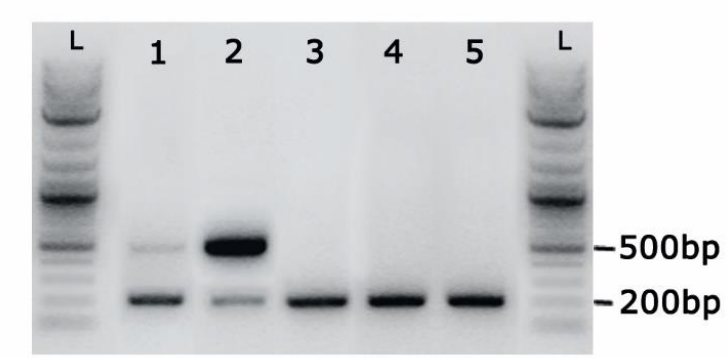
For optimum separation we recommend using a 2% agarose gel with TAE or TBE buffer used for electrophoresis.

Since the XpressMyco PCR Master Mix already contains a loading buffer, the samples can be applied directly to the gel after completion of the PCR program. The tracking dyes included allow visualization of the sample when loading the gel and can estimate the progress of electrophoresis — the yellow dye migrates with the running front.

Analysis

PCR template	PCR product	Result
Cell culture supernatant with internal control	500 bp and 200 bp	Mycoplasma contamination
	200 bp only	No contamination
	500 bp only	Severe Mycoplasma contamination (see troubleshooting)
	no band	PCR inhibitors present (see troubleshooting)
Control reaction without template	200 bp	Reagents are OK
	Any band	Contamination of the reagents (see troubleshooting)

Example of gel:



2% Agarose gel (TAE)

L: DNA ladder

Lane 1+2: contaminated cells

Lane 3+4: no infection with mycoplasma

Lane 5: control reaction without template

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Explanatory Remarks

1. It is important to check that mycoplasmas have been completely eliminated after each use of a mycoplasma removal kit to prevent the establishment of resistance. As resistance can be built up in the same way as in all use of antibiotics, complete elimination of mycoplasmas is vital.
2. First check whether at least two passages without the use of a mycoplasma removal kit were conducted between the last use and the actual test with XpressMyco Master PCR Detection Kit. If this was not the case, dead mycoplasmas may have been detected by the highly sensitive XpressMyco Master PCR detection kit.
3. The animal products used in cell culture are primary sources of mycoplasma contamination. To avoid this risk, use only fetal bovine serum (FBS) and trypsin that are guaranteed mycoplasma free.
4. Mycoplasmas belong to the class of *Mollicutes* and thus lack cell walls; they are resistant to many antibiotics that attack cell wall synthesis. The user is thus an important source of contamination in routine use of this type of antibiotic for cell culture. In this case, non-sterile working conditions go unnoticed, as the addition of antibiotics prevents the growth of most bacteria – and thus macroscopic effects – while allowing mycoplasmas to multiply unhindered.
5. Cross-contamination from another cell culture is possible. For this reason, always test all cultured cells and replace any potentially contaminated cell culture material (medium, FBS, trypsin, buffer).

Troubleshooting

1. **No PCR product for the internal control reaction:**

   This indicates that the sample contains PCR inhibitors:

- Do not use a cell culture supernatant of densely grown cells since this PCR may contain inhibitors.
- The sample should also not include cells or cell debris, since these can inhibit the PCR. The concentration of mycoplasmas in the cell culture supernatant is sufficient to be detected at this level of sensitivity.
- If no product is visible on the gel for the internal control reaction, we recommend isolating the genomic DNA from the cell supernatant using commercially available kits and then using this clean DNA as template.
- If no PCR product can be detected for the internal control reaction, but the gel shows a strong 500bp band, this is a clear indicator of high contamination by mycoplasmas. Due to the high concentration, the mycoplasma genome almost completely occupies the binding site at the active site of the polymerase, so that the internal control cannot be detectable amplified.

2. **Low signals:**

- Make sure that the cell culture supernatant is used from cell cultures covering 90% of the growth surface.

3. **Additional bands in the control reaction:**

   If the control reaction shows bands other than those attributable to primer dimers (“cloud” below 100bp) the reason might be contamination of the Master Mix or the water:

- Repeat the PCR run using fresh nuclease-free water. If bands are still detected, the Master Mix is contaminated and therefore unusable.