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XpressMyco PCR Detection Kit

Catalog No.: XMYC3-50

Description

The XpressMyco PCR Detection Kit enables you to detect even minor mycoplasma contamination in your cell cultures. The PCR primers can detect all relevant strains within supernatants of cultured cells. In addition to the primers, the set contains a hot-start Taq-polymerase, an optimized buffer solution with $MgCl_2$, deoxynucleotides and an internal control template. The PCR product length of this internal control is 700 bp and is used to confirm the polymerase-mediated amplification in all PCR samples. Contaminated cultures will show a single 500 – 520 bp amplification product depending on the mycoplasma strain.

Specifications

Application	PCR kit for detection of mycoplasmas in cell culture
Contents	Taq polymerase, Primer Mix, Internal control and Taq polymerase
Assays	50 applications per kit
Sensitivity	> 50 cfu/ml mycoplasmas
Shipping	Ice packs
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

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 min.
3. Spin the sample at 13,000 x g for 5 min to remove cell debris.
4. Use 2 µl of the supernatant as the template for the PCR.

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PCR Preparation

For optimum reliability, we recommend executing every PCR sample with the internal control template. When calculating the number of reactions, plan to conduct a reaction with internal control only, as well as a negative control using water instead of the templates.

The internal control confirms the absence of PCR inhibitors and rules out false positive results.

The negative control gives confidence that none of the reagents has been contaminated with genetic material.

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

Components	**Test sample and internal control**	**Test sample without internal control**	**Internal control only**	**Negative control**
Water	9.3 µl	10.3 µl	11.3 µl	12.3 µl
Taq polymerase buffer	2.7 µl	2.7 µl	2.7 µl	2.7 µl
Primer mix	9.0 µl	9.0 µl	9.0 µl	9.0 µl
Internal control	1.0 µl	–	1.0 µl	–
Taq polymerase (1 U/µl)	1.0 µl	1.0 µl	1.0 µl	1.0 µl
Test sample	2.0 µl	2.0 µl	–	–
Final volume	25.0 µl	25.0 µl	25.0 µl	25.0 µl

PCR Program

The following program yields optimal amplification of the 700 bp (internal control) and the 500 bp PCR product from different mycoplasma species.

Temperature (°C)	**Time (seconds)**	**Function**	**Number of cycles**
94	60	Pre-denaturation	1 cycle
94	30	Denaturation	35 cycles
62	30	Annealing	
72	60	Polymerization	
72	180	Final extension	1 cycle

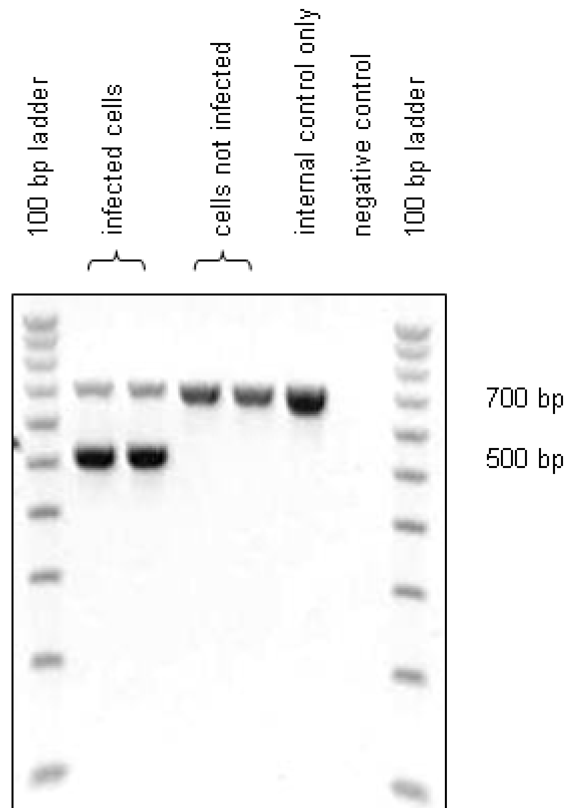
Electrophoresis of the PCR Products

For optimum separation, we recommend using a 2% agarose gel for electrophoresis.

Analysis

PCR template	PCR product	Result
Cell culture supernatant with internal control	500 bp and 700 bp	Mycoplasma infection
	700 bp only	No infection
	500 bp only	Severe Mycoplasma infection
	no band	PCR inhibitors present (see troubleshooting)
Cell culture supernatant	500 bp	Mycoplasma infection
	No band	No infection if "internal control" was successful
Internal control	700 bp only	PCR successful
	500 bp and 700 bp	Contamination of the reagents
	No band	PCR inhibitors present (see Troubleshooting)
Negative control	No band	reagents are ok
	Any band	Contamination of the reagents (see Troubleshooting)

Example of gel (all samples with internal control):



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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 PCR detection kit. If this was not the case, dead mycoplasmas may have been detected by the highly sensitive XpressMyco 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. In addition, 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. Internal control-only reactions should produce a single 700 bp band.

   Additional bands indicate contamination of one or more PCR components:

   - 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.
2. **No PCR product in the internal control** reaction indicates that the sample contains PCR inhibitors:
   - The templates should not contain cells, as them can inhibit PCR. The concentration of mycoplasma in the cell culture supernatant is sufficient for a sensitive PCR examination.
3. **Low signals** indicate poor amplification efficiencies of mycoplasma.
   - Regrow the cells and harvest the supernatants from cultures where the cells cover 90% of the growth surface. Repeat the PCR using these new samples.