MycoSPY®

Mycoplasma PCR Detection Kit

For ordering information and SDS see www.biontex.com

Product	Order No.	Size
MycoSPY®	M030–050	50 assays

Shipping: Ice packs

Storage: ≤ -15°C, avoid multiple freeze-thaw cycles

Stability: Best before: see label

Use: Only for research purposes *in vitro*; not intended for human or animal diagnostic, therapeutic or other clinical uses.

Description

The PCR-based mycoplasma detection kit MycoSPY® 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 additional 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.

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1. General Remarks

1.1 Specifications

Application	PCR kit for detection of mycoplasmas in cell culture
Contents	Taq polymerase, Primer Mix, Internal control and Taq polymerase buffer with nucleotide triphosphates (dNTPs)
Assays	50 applications per kit
Sensitivity	>100 copies of the mycoplasma genome under optimal conditions (highly pure M. fermentans DNA, without internal control)
Shipping	ice packs
Storage	≤ 15°C

1.2 General Considerations

The contamination of cell cultures by mycoplasma remains one of the major problems in biological research. Most mycoplasma contaminations occur due to the use of animal products like sera or trypsin. Another possible source of contamination is the laboratory personnel itself.

In order to protect cell cultures against mycoplasma effectively, a routine examination of the cell culture and a removal of the mycoplasma is necessary. The Biontex products MycoSPY® (detection) and MycoRAZOR® (removal) are ideal for these tasks.

1.3 Contaminations

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.

1.4 List of *Mollicute* strains detected with MycoSPY®

About a quarter of all animal cell cultures are contaminated with Mycoplasma/Mollicutes. The most commonly found strains in cell cultures with a total probability of 94% are: M. fermentans (47%), M. hyorhinis (19%), M. orale (10%), M. arginini (9%), A. laidlawii (6%) and M. hominis (3%). In addition, the following strains were found with lower probability: M. gallisepticum, M. pneumoniae, M. salivarium, M. synoviae and S. citri. All of these Mollicutes strains are detected by MycoSPY® in addition to a variety of other strains. The verification of the primer specificity was carried out by BLAST analysis.

Detection range

The list shows only Mollicute strains with 100% primer match:

Mycoplasma				
M. agalactiae	M. citelli	M. genitalium	M. molare	M. pneumoniae
M. alligatoris	M. columborale	M. hominis	M. mucosicanis	M. pulmonis
M. alvi	M. conjunctivae	M. hyopneumoniae	M. muris	M. salivarium
M. amphoriforme	M. cricetuli	M. hyorhinis	M. mustelae	M. sualvi
M. arginini	M. crocodyli	M. hyosynoviae	M. mycoides	M. synoviae
M. bovigenitalium	M. cynos	M. imitans	M. orale	M. testudineum
M. bovis	M. dispar	M. iowae	M. oxoniensis	M. testudinis
M. buccale	M. edwardii	M. lacerti	M. penetrans	M. verecundum
M. canadense	M. felis	M. lagogenitalium	M. phocidae	M. volis
M. canis	M. fermentans	M. microti	M. phocicerebrale	M. yeatsii
M. capricolum	M. gallisepticum	M. moatsii	M. pirum	M. zalophidermidis
Ureaplasma				
U. canigenitalium	U. diversum	U. gallorale	U. parvum	U. urealyticum
Mesoplasma				
M. chauliocola	M. florum	M. photuris	M. tabanidae	
M. entomophilum	M. grammopterae	M. syrphidae		
Spiroplasma				
S. cantharicola	S. citri	S. lineolae	S. platyhelix	S. taiwanense
Acholeplasma				
A. laidlawii				

2. User Protocol

2.1 Sample preparation

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

The cells should have been subcultured regularly (every 2-3 days) and cover about 90% of the growth surface when the cell culture supernatant is harvested!

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.

2.2 Preparation of the PCR

Preparation of the MycoSPY® reagents

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

Preparation of the PCR mixture

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

The reason for the decrease in sensitivity is that the internal control uses the same primers as the mycoplasma genome. Normally this is not a problem, as mycoplasmas multiply very quickly, so that a contaminated cell culture reaches a high concentration of mycoplasma genome in a short time. However, the disadvantage is that contamination at an early stage with a very low mycoplasma concentration is not detected. To obtain full sensitivity, we recommend testing the sample in two individual tubes, one with and one without internal control.

The negative control shows that the reagents have not been contaminated with genetic material.

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

2.3 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

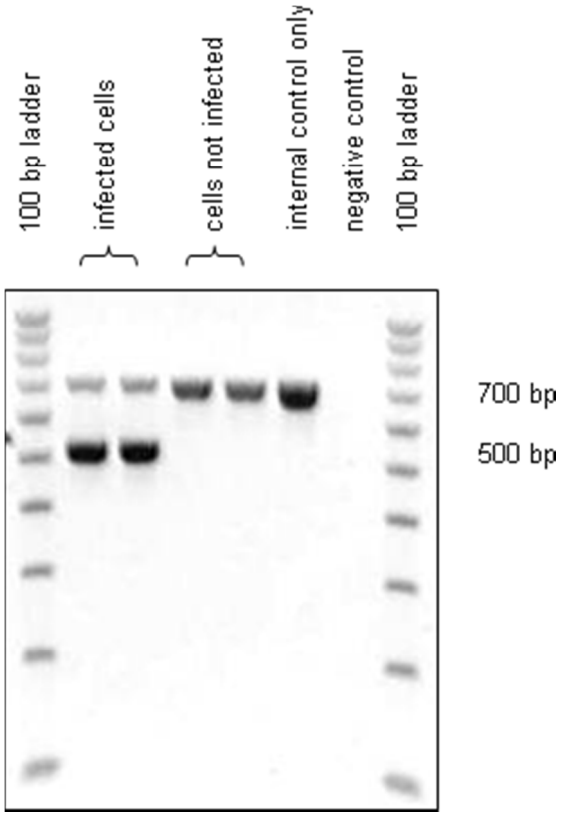
2.4 Electrophoresis of the PCR Products

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

2.5 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):



2.6 Explanatory remarks

1. It is important to check that mycoplasmas have been completely eliminated after each use of a mycoplasma removal kit (e.g. MycoRAZOR®) 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 MycoSPY®. If this was not the case, dead mycoplasmas may have been detected by the highly sensitive MycoSPY®.
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).

3. 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.

4. Miscellaneous

4.1 Important Information

This reagent is developed and sold for research purposes and *in vitro* use only. It is not intended for human or animal therapeutic or diagnostic purposes.

MycoSPY® is a registered trademark of Biontex Laboratories GmbH.

4.2 Warranty

Biontex guarantees the performance of this product until the date of expiry printed on the label when stored and used in accordance with the information given in this manual. If you are not satisfied with the performance of the product please contact Biontex Laboratories GmbH.

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