

MycoSPY[®] qPCR Master Mix

with FAM/HEX fluorescent probes, lyophilized

qPCR Kit for the Detection of Mycoplasma

For ordering information and SDS see www.biont.com

Product	Order No.	Size
MycoSPY [®] qPCR Master Mix	M010-025	25 assays
	M010-050	50 assays (2 x 25)

Shipping: At ambient temperature

Storage: Lyophilized $\leq -15^{\circ}\text{C}$, Rehydrated $\leq -15^{\circ}\text{C}$, store away from light

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

MycoSPY[®] qPCR Master Mix is designed for routine testing of cell cultures for mycoplasma. There is no need for time-consuming isolation of genomic DNA - a sample of the cell culture supernatant is used as a template for qPCR. A few pipetting steps for the qPCR setup and real-time analysis deliver fast, simple, and highly sensitive results.

Index

1. General Information	2
1.1 Specifications of the MycoSPY® qPCR Master Mix	3
1.2 Explanatory remarks	3
2. User Protocol	4
2.1 Workflow	4
2.2 Sample preparation	4
2.3 Setup of the reaction	5
Preparation of the MycoSPY® qPCR Master Mix	5
Preparation of the PCR mixture	5
2.4 PCR program	6
2.5 Real-Time Detection of the PCR products	6
2.6 Analysis	7
3. Troubleshooting	8
4. Appendix	8
4.1 List of <i>Mollicute</i> strains detected by MycoSPY® qPCR Master Mix	8
Detection range	9
5. Miscellaneous	9
5.1 Important information	9
5.2 Warranty	9

1. General Information

The contamination of cell cultures with mycoplasma is one of the main problems in cell biology research. It is therefore essential to regularly test cell cultures for mycoplasma. Biontexas offers the MycoSPY® series of products for detection and MycoRAZOR® for elimination.

The qPCR-based MycoSPY® qPCR Master Mix mycoplasma detection kit enables reliable, specific, and rapid detection of mycoplasma contamination in cell cultures.

The primers contained in the Master Mix detect all *Mollicute* strains relevant to cell culture. In addition to the primers, the Master Mix contains the components required for qPCR (buffer, Taq polymerase, dNTPs, fluorescence-labeled oligonucleotide probes). A FAM oligonucleotide probe detects the *Mollicute* strains. Depending on the strain, cultures contaminated with mycoplasma produce a PCR product that can be detected in real-time in the FAM channel using a suitable qPCR thermocycler.

The light protected lyophilized form of MycoSPY® qPCR Master Mix is stable at room temperature, thereby reducing packaging costs for shipping the product.

The kit also includes an Internal Control that produces an approx. 200 bp PCR product and is detected with a HEX oligonucleotide probe. This control allows the presence of PCR inhibitors and thus false negative results to be ruled out. PCR-grade water is included in the kit for rehydrating the lyophilized master mix.

1.1 Specifications of the MycoSPY® qPCR Master Mix

Application	qPCR kit for routine screening of cell cultures for mycoplasma
Content	<ul style="list-style-type: none">• Lyophilized Master Mix: HotStart Taq-polymerase, primer mix, nucleotide triphosphates (dNTPs), buffer, MgCl₂, stabilizers, FAM and HEX oligonucleotide probes• 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	>100 copies of the mycoplasma genome under optimal conditions (highly pure <i>M. fermentans</i> DNA, without Internal Control)
Shipping	At ambient temperature
Storage	≤ 15°C (store away from light)

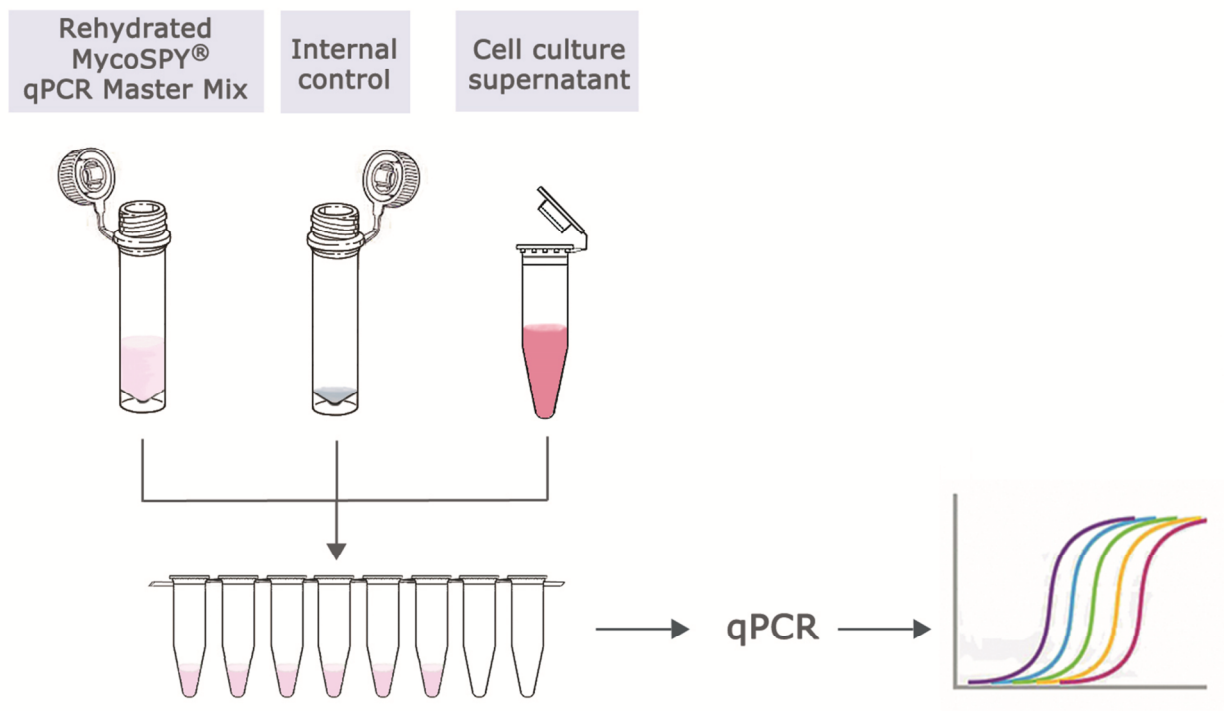
1.2 Explanatory remarks

1. A qPCR thermocycler capable of detecting the fluorescent dyes FAM and HEX is required for the correct use of this kit.
2. First, check that at least two normal cell culture passages have been performed between the last use of a mycoplasma removal kit and the current test with the MycoSPY® qPCR Master Mix Kit. If this is not the case, DNA from dead mycoplasma could still be detected due to the very high sensitivity of the MycoSPY® qPCR Master Mix.
3. Cross-contamination from other cell cultures is common. Therefore, always test all cells in culture and replace any potentially contaminated cell culture material (medium, FBS, trypsin, buffer).
4. 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. Complete elimination of mycoplasmas is vital as resistance can be built up in the same way as in all use of antibiotics.
5. A major source of mycoplasma contamination is animal products used in cell culture. Therefore, only use fetal bovine serum (FBS) and trypsin that are guaranteed to be free of mycoplasma.
6. Since mycoplasma belong to the *Mollicutes* class and therefore have no cell wall, they are resistant to many antibiotics that target the synthesis of bacterial cell walls. The user is a potential source of contamination when such antibiotics are used as standard in cell culture. Unsterile working practices and the associated introduction of mycoplasma go unnoticed in this case, as the added antibiotic prevents the growth of most bacteria – and thus macroscopic signs. However, mycoplasma can still multiply here.

2. User Protocol

Wear gloves while preparing the samples and qPCR to avoid false positive results. To prevent cross-contamination between samples, we recommend using filter tips for pipetting. Please note that the fluorescent dyes are very sensitive to light. Therefore, **consistently protect the master mix from light exposure** (e.g., during pipetting with aluminum foil and during storage with the opaque original packaging). Keep the standing time in liquid form as short as possible while working.

2.1 Workflow



2.2 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 pelletize cell debris.
4. Use 2 µl of the supernatant as the template for the PCR.

2.3 Setup of the reaction

Preparation of the MycoSPY® qPCR Master Mix

Prior to first use, the lyophilized Master Mix must be rehydrated. For this purpose, pipette 575µl of water (tempered to room temperature, water included in the kit) to the lyophilisate and dissolve completely by inverting the tube several times. This amount is sufficient for 25 reactions. If not used up completely, the rehydrated Master Mix **must be stored immediately after use at ≤ -15°C and protected from light**, as a long standing time in a liquid state at room temperature has a negative effect on sensitivity. Repeated freezing and thawing, however, is possible without any problems.

Preparation of the PCR mixture

For optimum reliability, we recommend performing each PCR reaction 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 rules out 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 because mycoplasma multiplies 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 in the early stages with very low mycoplasma concentrations is not detected. If you want to maintain full sensitivity, we recommend testing the sample in two separate tubes, one with and one without the Internal Control.

Include an additional reaction without template. This control reaction ensures that there is no contamination of the reaction components with genetic material.

Transfer the following volumes to individual qPCR tubes:

Components	Test sample with Internal Control	Control reaction
Master Mix	22 µl	22 µl
Internal Control	1 µl	1 µl
Test sample	2 µl	-
Water	-	2 µl
Final volume	25 µl	25 µl

2.4 PCR program

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

Temperature (°C)	Time (s)	Function	Number of cycles
95	120	Initial denaturation and activation of Taq polymerase	1 cycle
94	5	Denaturation	45 cycles
65	25	Primer-Annealing & Elongation	
4	∞		

Depending on the thermocycler, the program takes between 1 and 1.5 hours.

Expected products:

Amplificate of the Internal Control in the HEX channel.

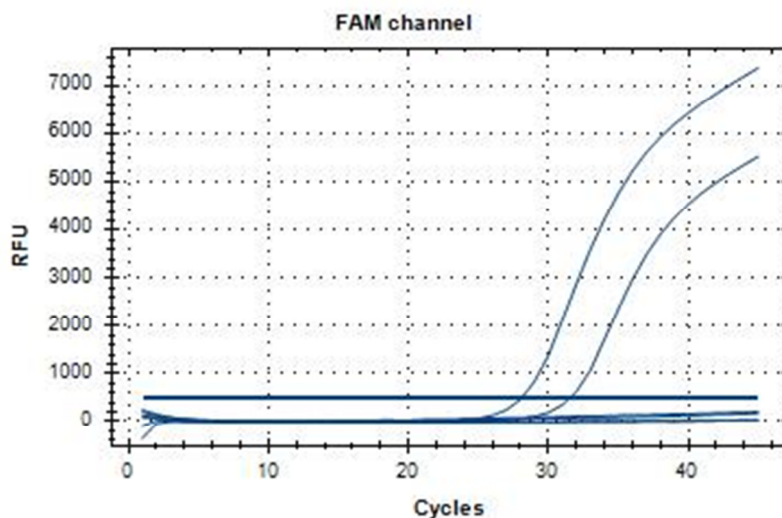
Amplificate of the mycoplasma genome in the FAM channel.

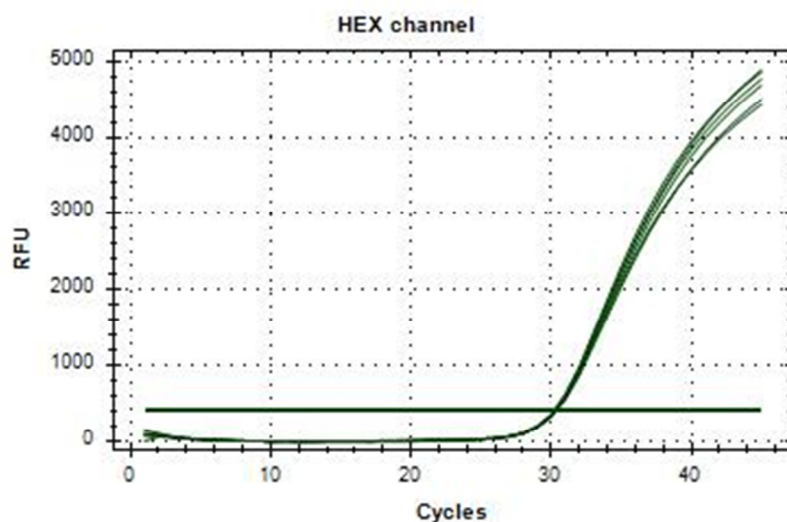
2.5 Real-Time Detection of the PCR products

Save the data generated by the thermocycler at the end of the program run. Read out the FAM and HEX channels. When determining the Ct values, follow the standard routine of your cycler or set the threshold yourself. Check the amplification curves for their typical exponential rise (see also Troubleshooting). Evaluate the Ct values for the Internal Control (HEX channel), negative control (HEX & FAM channels), and for samples with mycoplasma contamination (FAM channel).

Example:

Amplification of 6 samples from cell cultures, each with Internal Control. 2 samples are infected with mycoplasma (see FAM channel). 6 signals can be seen in the HEX channel, i.e., there is no PCR inhibition (Bio-Rad CFX Duet qPCR System).





2.6 Analysis

The presence of mycoplasma is indicated by an increasing fluorescence signal in the FAM channel. Quantification is also possible, but a dilution series (of known concentration) of the detected *Mollicute* strain must be used in the same run. Successful PCR without inhibition is indicated by an increasing fluorescence signal in the HEX channel:

PCR template	FAM channel	HEX channel	Result
Cell culture supernatant	Signal at Ct<40	Signal at Ct<35	Mycoplasma contamination
	No signal	Signal at Ct<35	No contamination
	No signal	No signal or signal at Ct>35	PCR inhibitors present (see troubleshooting)
Control reaction without template	No signal	Signal at Ct<35	Reagents are OK
	Signal	Signal at Ct<35	Contamination of the reagents (see troubleshooting)

3. Troubleshooting

1. No signal or signal at Ct>35 in the HEX channel for Internal Control:

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, despite these measures, no signal or a signal with Ct>35 cannot be detected for the Internal Control in the HEX channel, it is recommended to isolate the genomic DNA using commercially available kits and to use this purified DNA as a template.
- If no signal for the Internal Control can be detected in the HEX channel, but a signal with a low Ct value (<20) can be detected in the FAM channel, there is a very high level of mycoplasma contamination. Due to the high concentration, the mycoplasma genome almost completely occupies the binding site at the active center of the polymerase, so that the Internal Control cannot be amplified in a detectable manner.

2. Unusual amplification curves in the FAM channel at Ct>40:

In rare cases, false positive signals may occur. These usually show an unusual amplification curve and signals with high Ct values >40. In these cases, the qPCR should be repeated.

4. Appendix

4.1 List of *Mollicute* strains detected by MycoSPY® qPCR Master Mix

About a quarter of all animal cell cultures are contaminated with mycoplasma/*Mollicutes*. The strains most commonly found 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 these *Mollicute* strains, in addition to a variety of other strains, are detected by MycoSPY® qPCR Master Mix. BLAST analysis was used to verify the primer specificity.

Detection range

The list shows only *Mollicute*-strains with a 100% primer and probe 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. bovis	M. cynos	M. imitans	M. orale	M. testudineum
M. bovigenitalium	M. dispar	M. iowae	M. oxoniensis	M. testudinis
M. bovis	M. edwardii	M. lacerti	M. penetrans	M. verecundum
M. buccale	M. felis	M. lagogenitalium	M. phocidae	M. volis
M. canadense	M. fermentans	M. microti	M. phocicerebrale	M. yeatsii
M. canis	M. gallisepticum	M. moatsii	M. pirum	M. zalphiddermidis
M. capricolum				
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				

5. Miscellaneous

5.1 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.

5.2 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.

Biontex Laboratories GmbH
Landsberger Straße 234
im MGH
80687 München/Laim
Germany

Tel.: +49 (0)89 3247995-0
Fax: +49 (0)89 3247995-2
E-Mail: contact@biontex.com
Internet: www.biontex.com