

MycoSPY® Master Mix

including loading buffer and tracking dye, lyophilized

PCR kit for the detection of mycoplasma

For ordering information and SDS see www.biont.com

Product	Order No.	Size
MycoSPY® Master Mix	M020-025	25 assays
	M020-050	50 assays (2 x 25)

Shipping: At ambient temperature

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

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 MycoSPY® Master Mix is designed for the routine examination of cell cultures on 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.

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1. General Remarks

The contamination of cell cultures by mycoplasma remains one of the major problems in cell biology research. Routine examination of the cell culture is necessary in order to identify mycoplasma infection effectively. The Biontex product series of MycoSPY® (detection) and MycoRAZOR® (removal) are ideal for these tasks.

The PCR-based mycoplasma detection kit MycoSPY® Master Mix enables contamination of cell cultures with mycoplasmas to be detected reliably, specifically, and quickly.

The PCR primers included in the Master Mix detect all *Mollicute* strains relevant for cell culture. Additionally the Master Mix contains all other PCR components required (buffer, Taq-polymerase, dNTPs). A loading buffer and two tracking dyes in the Master Mix allow the PCR reaction to be loaded directly onto an agarose gel. Depending on the strain, mycoplasma-contaminated cultures show a single 500-520 bp PCR product, which is easily detected on the agarose gel.

The lyophilized form of the MycoSPY® Master Mix is stable at room temperature and therefore reduces the amount of packaging for the shipment of the product.

The kit includes an internal control that enables the presence of PCR inhibitors, and thus false negative results, to be excluded. The kit includes PCR-suitable water for rehydration of the lyophilized Master Mix.

1.1 Specifications of the MycoSPY® Master Mix

Application	PCR 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 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	>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

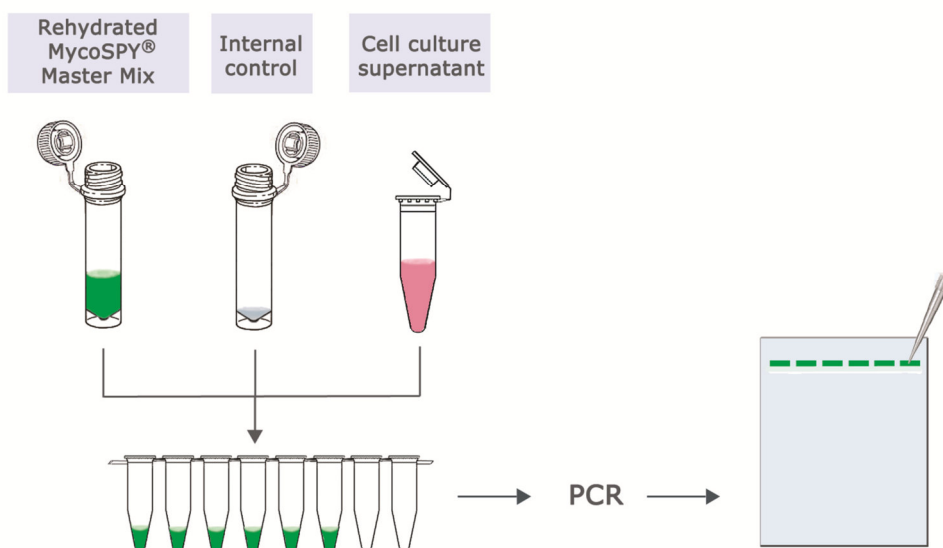
1.2 Explanatory remarks

1. First check whether at least two passages without the use of a mycoplasma removal kit were conducted between the last use and the current test with MycoSPY® Master Mix. If this was not the case, dead mycoplasmas may have been detected by the highly sensitive MycoSPY® Master Mix.
2. Cross-contamination from other cell cultures is possible. For this reason, always test all cultured cells and replace any potentially contaminated cell culture material (medium, FBS, trypsin, buffer).
3. 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.
4. 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.
5. 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.

2. User Protocol

To avoid false positive results, wear gloves while preparing the templates and the PCR. To avoid cross-contamination between samples, we recommend using aerosol-resistant pipet tips throughout the whole protocol.

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® 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 at ≤ -15°C immediately after use**, 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 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. If you want to obtain full sensitivity, we recommend testing the sample in two individual tubes, one with and one without 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 PCR 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
94	60	Initial denaturation and activation of Taq polymerase	1 cycle
94	30	Denaturation	35 cycles
62	30	Annealing	
72	30	Polymerization	
72	180	Final elongation	1 cycle
4	∞		

Expected products:

~200 bp Internal Control

~500 bp amplicon of the mycoplasma genome

2.5 Electrophoresis of the PCR products

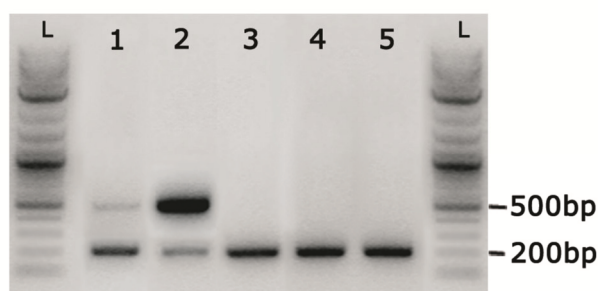
For optimum separation we recommend using a 2% agarose gel with TAE or TBE buffer used for electrophoresis.

Since the MycoSPY® 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.

2.6 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 a 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

3. 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.

4. Appendix

4.1 List of *Mollicute* strains detected by MycoSPY® 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® Master Mix. BLAST analysis was used to verify of the primer specificity.

Detection range

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

Mycoplasmas				
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. suis
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. buccale	M. edwardii	M. lacerti	M. penetrans	M. verocellum
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. zootrichum
Ureaplasmas				
U. canigenitalium	U. diversum	U. gallorale	U. parvum	U. urealyticum
Mesoplasmas				
M. chauliocola	M. florum	M. photuris	M. tabanidae	
M. entomophilum	M. grammopterae	M. syrphidae		
Spiroplasmas				
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.

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