Mycoplasma Detection Kit

Cat#: orb533482 (User Manual)

Description

Mycoplasma Detection Kit provides a highly sensitive, easy-to-perform and rapid tool for detection of mycoplasma contaminations in cell cultures or other biological materials. The kit is based on the amplification of a conserved 16S rRNA coding region of Mycoplasma by PCR resulting in a characteristic 268 bp fragment. It allows the detection of common avian, bovine, porcine and human Mycoplasma and Ureaplasma species with extreme sensitivity. Due to this sensitivity, please pay special attention to avoid cross contaminations.

Table 1: Tested species

Species	Origin
Mycoplasma bovis	**Bovine**
Mycoplasma columborale	**Avian**
Mycoplasma bovigenitalium	**Bovine**
Mycoplasma iners	**Avian**
Mycoplasma gallinarum	**Avian**
Mycoplasma faucium	**Human**
Mycoplasma gallinaceum	**Mammalian/Avian**
Mycoplasma hominis	**Human**
Mycoplasma hyorhinis	**Porcine**
Mycoplasma synoviae	**Avian**
Ureaplasma urealyticum	**Human**

Kit contents

Hot Start Polymerase (red cap) S pack: 7 µl
L pack: 30 µl

Master Mix (green cap) S pack: 250 µl
L pack: 1.25 ml

Control DNA (white cap) S pack: 7 µl
L pack: 30 µl

Sample Buffer (blue cap) S pack: 600 µl
L pack: 3 ml

Additionally required material
- pipettes and filter tips
- PCR tubes
- micro centrifuge
- PCR thermal cycler
- agarose gel and electrophoresis system

Protocol

Preparation of cell culture supernatant
Transfer 0.5 to 1 ml supernatant immediately prior to splitting of the cells to a sterile vial. Growing the cells without antibiotics is not necessary.
- centrifuge samples for 30 sec at 250 x g
- transfer supernatant in a new vial and discard cell debris
- centrifuge for 15 min at 13.000-15.000 x g to sediment the mycoplasma
- decant carefully and discard supernatant
- resuspend the pellet (please note that the pellet may not be always visible) in 50 µl Sample Buffer and vortex well
- incubate the samples for 5 min at 95°C
- centrifuge the samples briefly and place them on ice

PCR program

Temperature	Time	Number of Cycles
94°C	2 min	1
94°C	30 sec	35
55°C	30 sec	
72°C	30 sec	
72°C	2 min	1

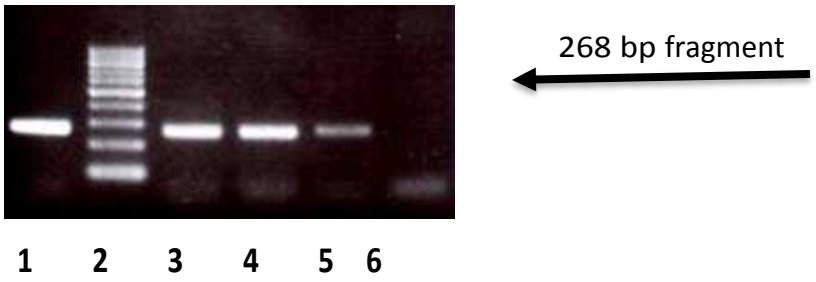
Analysis of amplified products

- add 5 µl gel loading buffer to each vial, centrifuge and mix briefly
- load 5 µl of each assay onto a 2 % agarose gel and run gel electrophoresis

Preparation of other biological material

Testing of mycoplasma contaminations in sera, cryo cultures or cells requires the extraction of DNA prior to PCR. The use of a genomic DNA Extraction Kit is recommended.

Gel analysis



1: positive control
2: 100 bp DNA Ladder
3 and 4: strongly contaminated samples 5: weakly contaminated sample
6: negative control

PCR reaction

Prepare a Premix of the following components:

Premix	1 sample	5 samples
Master Mix	**23.5 µl**	**117.5 µl**
Polymerase	**0.5 µl**	**2.5 µl**

For each assay pipet 24 µl Premix in a PCR vial and add 1 µl of the prepared sample. For preparation of the positive control add 1 µl of Control DNA, as negative control apply 1 µl Sample Buffer. Mix and centrifuge the vials briefly. Place the vials in a thermocycler.