SouthernBiotech

Mycoplasma Detection Kit
User Guide

Cat. No. 13100-01
50 Reactions
For Research Use Only

Description

The SouthernBiotech Mycoplasma Detection Kit is designed to specifically detect potential *Mycoplasma* contamination in cell cultures. The kit incorporates polymerase chain reaction (PCR) to amplify the conserved 16S ribosomal RNA coding region within the *Mycoplasma* genome, thereby providing an extensive, highly sensitive, and efficient detection method. Carefully determined primer sequences cover three genera of Mycoplasmatales (*Mycoplasma*, *Acholeplasma*, and *Ureaplasma)* which allows the kit to detect over 95% of potential cell culture infections.

The PCR technology in the Mycoplasma Detection Kit is fast (results are typically obtained in less than 3 hours) and easy to use. The kit is also highly sensitive and can detect as little as 2-5 femtograms of *Mycoplasma* DNA in 100 µL of test sample supernatant. Eukaryotic and bacterial DNA from cell culture supernatant is not amplified by the kit.

A sample cell line infected with *Mycoplasma* will generate a PCR product between ~448 bp to ~611 bp on an agarose gel, depending on the type of *Mycoplasma* present. A positive control (*M. orale*, 503 bp) is included to validate that the PCR amplification process has occurred as well as to confirm the size of the PCR product obtained in test samples. An internal control is also provided to eliminate potential false negatives associated with PCR inhibitors.

Kit Components

- User guide
- Primer set and nucleotides (lyophilized) - Blue Cap
  - Primer/dNTP mix (deoxynucleotide triphosphates including dATP, dCTP, dGTP, and dUTP)
- Sterile PCR 10X reaction buffer (500 µL) - White Cap
  - 100 mM Tris-HCl, pH 8.5
  - 750 mM KCl
  - 30 mM $MgCl_2$
- Positive Control DNA (lyophilized) - Yellow Cap
  - Non-infectious PCR product from *M. orale*
  - Yields a 503 bp band
- Internal Control DNA (lyophilized) - Green Cap
  - Non-infectious plasmid DNA including *Mycoplasma*-specific primer sequences
  - Yields a 270 bp band

Required Materials

- Thermal cycler
- Taq DNA polymerase
  - The following protocol has been optimized for use with Hot Start Taq DNA polymerase
  - The use of other DNA polymerases is not recommended
- DNA ladder
- PCR reaction tubes
- DNase free water

Strains Amplified

Strain	Base Pairs (bp)
A. laidlawii	611
M. arginini	448
M. arthritidis	486
M. bovis	605
M. cloacale	459
M. falconis	458
M. faucium	479
M. fermentans	579
M. genitalium	464
M. hominis	448

Strain	Base Pairs (bp)
M. hyorhinis	525
M. hyosynoviae	471
M. opalescens	573
M. orale	503
M. pirum	533
M. pneumonia	493
M. salivarium	482
M. synoviae	572
U. urealyticum	558

Handling and Storage

The kit is stable for the period shown on the label when stored as directed. Upon reconstitution of the primer/dNTP mix, the positive control, and the internal control, store below -20°C. Avoid freeze / thaw cycles.

Suggested Protocol

- Reagent Preparation
  - Centrifuge all kit component tubes to ensure that material has completely settled to the bottom
  - Reconstitute primer/dNTP mix, positive, and internal control using DNase free water as follows -
    - Primer/dNTP mix - 260 µL
    - Positive control - 200 µL
    - Internal control - 200 µL
  - Vortex for 5 seconds to ensure thorough mixing and equilibrate at room temperature for 5 minutes
  - Vortex, then centrifuge again
  - Reconstituted reagents should be stored at or below -20°C; avoid multiple freeze / thaw cycles

- Sample Preparation
  - Transfer 100 µL of cell culture supernatant* to a sterile 200 µL PCR tube; ensure lid has been tightly sealed to prevent evaporation
  - Heat the sample supernatant at 95°C for 5 minutes
  - Quickly spin the sample supernatant at maximum speed for 5 seconds to remove cell debris; the supernatant is now ready to add to the PCR master mix

    *This kit is specifically designed for *Mycoplasma* detection from cell culture supernatant. For serum or cell lysate samples, DNA extraction is recommended prior to testing to avoid PCR inhibition.

- Thermal Cycling Program
  - Incubation times are dependent on the type of polymerase used; the template described below has been optimized for Hot Start Taq polymerase
    - 1 cycle — 95°C for 5 minutes
    - 35 cycles — 95°C for 30 seconds
58°C for 40 seconds
72°C for 1 minute
    - Cool down to 4°C

- PCR Master Mix
  - Total volume per reaction is 50 µL
    - When preparing reactions, calculations should also include positive and negative controls
    - Add 48 µL of master mix to each tube
    - Contents of master mix are provided below -

Component	1 Reaction (µL)	10 Reactions (µL)	50 Reactions (µL)
Water	35.6	356	1780
10X reaction buffer	5	50	250
Primer/dNTP mix	5	50	250
Internal control	2	20	100
Taq polymerase	0.4	4	20

Note - If using a polymerase other than Taq, use the PCR buffer supplied with the enzyme and the final $MgCl_2$ concentration must be adjusted to 3.0 mM

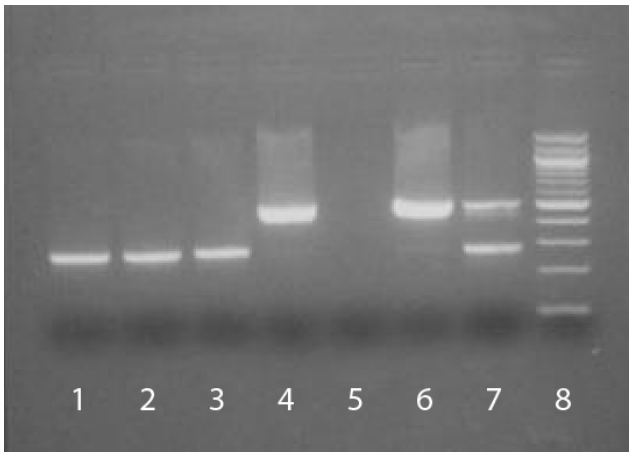
  - Add 2 µL of DNase free water as a negative control into the appropriate PCR reaction tube
  - Add 2 µL of reconstituted positive control DNA supplied in the kit into the appropriate PCR reaction tube
  - Add 2 µL of heat-treated test sample into the appropriate PCR reaction tube
  - Run according to the thermal cycling program

- Agarose Gel Electrophoresis
  - Use a 1% - 1.5% agarose gel containing 0.5 µg/mL ethidium bromide
  - Load 15 µL - 20 µL of each PCR reaction mixed with bromophenol blue loading buffer into each well
  - Run at 100 V for 20 min
  - Detect PCR product bands using UV box

    Note - Ethidium bromide is a known mutagen and should be handled as a hazardous chemical; wear gloves when handling

- Gel Evaluation
    - Samples containing *Mycoplasma* infection will contain a band (or multiple bands) between ~448 bp to ~611 bp
    - Samples containing the supplied internal control DNA will contain a distinct 270 bp PCR product that is clearly distinguishable from the ~448 bp to ~611 bp band(s) found in positive samples (see sample data, lane 7)
        - The internal control indicates a successfully performed PCR reaction
        - PCR inhibition may have occurred if the internal control band disappears in some samples, but the band is present in the PCR reaction of the negative control (water added instead of test sample)
            - If the PCR of a sample is inhibited, the inhibitors can be easily removed by performing a DNA extraction
    - If the cell culture is heavily contaminated with *Mycoplasma*, amplification of the ~448 bp to ~611 bp product(s) may diminish or completely eliminate the 270-bp internal control product (see sample data, lane 6)
    - Low intensity bands smaller than 100 bp indicate the presence of non-specific, self-annealing primers; this does not indicate a positive result and will not affect the precision of the test

Sample Data



Lane 1	*E. coli* Genomic DNA	Lane 5	Inhibited Sample
Lane 2	Hybridoma Cell DNA	Lane 6	Strongly Contaminated Sample
Lane 3	Negative Control (H_20)	Lane 7	Weakly Contaminated Sample
Lane 4	Positive Control	Lane 8	100 bp DNA Ladder

References

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