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Product Information

SwiftDx Mycoplasma Detection Kit

CatLog Number: **FP1-Myc-AA**

Storage Temperature: 2-8 °C

Instructions For Use

Product Description

The SwiftDx Mycoplasma Detection Kit is a point of use screening test for mycoplasma contamination which uses a modified version of a polymerase chain reaction (PCR) with a labelled oligo system that allows mycoplasma PCR product to be detected on a lateral flow device.

The primer sets in the kit are specific to the highly conserved 16S rRNA coding region in the mycoplasma genome. This should allow detection for all mycoplasma species usually encountered as contaminants in cell culture. SwiftDx will be continually testing different species and adding them to the "confirmed detected" list on our website (swiftdx.co.uk).

SwiftDx use a modified PCR approach that ensures only the specific 16S rRNA amplicons are detected on the lateral flow test strip. The test provides a very clear positive result when contamination above the test limit of detection is present, i.e., 3 days post contamination. The SwiftDx Mycoplasma Detection Kit requires the use of cell culture supernatant. The sample requires no further processing. The use of a thermal cycler for the modified PCR method ensures denaturing of possible biological contaminants. The test is intended to be used at the point of cell culture use with the generation of results within an hour, without the need for third party testing.

Kit Components

- Lateral flow tests strips: 25 units
- Solution I: 200 µl
- Solution II: 200 µl
- Solution III: 400 µl
- Running Buffer: 10 ml
- Positive Control Sample: 25 µl
- Negative Control Sample: 25 µl

Equipment and Reagents Required but Not Provided

- Thermal Cycler: The SwiftDx Mycoplasma Detection Test Kit has been validated for use with Applied Biosystems SimpliAmp Thermal Cycler; however, any suitable alternative should pose no loss in performance. See 'Procedure' section below
- Amplification tubes, sterile and DNAse free
- 96-well U- or flat-bottomed plate or 1.5 ml tubes

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

This method is suitable for adherent cell culture methodologies and is not suitable for suspension methodologies.

Storage/Stability

Upon receipt of the kit please immediately store at 2–8 °C.

Procedure

A. Preparation of Sample Material

Cell lines should be pre-cultured in the absence of antibiotics for at least 3 days to maximise test sensitivity. Ideally, samples should be derived from cultures that are at 90–100% confluence. PCR inhibiting substances may accumulate in the medium of older cultures. However, results have been successfully derived from a range of cell culture growth stages.

To avoid incorrect results, the use of aerosol-preventive filter tips and gloves is recommended. The SwiftDx Mycoplasma Detection Kit sample can be taken directly from cell culture supernatants without prior preparation. However, cell media can be boiled (95ºC for 5 minutes) to denature potential mycoplasma cells if they are to be processed in non-contained environments. Samples can be frozen at -20 °C for future use.

B. Reaction Setup

Running a positive and negative control reaction with each set of tests is recommended. These samples are provided in the kit.

1. **Create a reaction mix by combining 5 µl of Solution I and 5 µl of Solution II in an amplification tube.**

   Before pipetting, ensure thorough mixing by flicking the tubes of **Solution I** and **Solution II**.

   A master mix of **Solution I** and **Solution II** can be created for the number of reactions being performed. For each reaction to be run, aliquot 12 µl of master mix into an amplification tube.

2. **Add 2 µl of cell culture supernatant to each reaction**.

   Mix thoroughly by pipetting up and down, or by flicking the tube followed by brief centrifugation.

C. Reaction

Run the 36 cycle programme

Create the following programme according to the manufacturer's instructions supplied with your cycler.

Thermal Cycler 36 cycles

1 cycle	95 ºC for 1 minute
36 cycles	95 ºC for 5 seconds 72 ºC for 12 seconds
1 cycle	72 ºC for 20 seconds

Cooldown to 4 ºC

1. Place reaction tubes into thermal cycler and run the program.
2. **Add 10 µl of Solution III to each reaction tube and place back in thermal cycler for the final cycle.**

   Place the reaction tubes back into the thermal cycler and run the following program:

   Thermal Cycler 1 cycle

1 cycle	95 ºC for 1 minute 72 ºC for 35 seconds

   Cooldown to 4 ºC

D. Run Sample on Test Strip

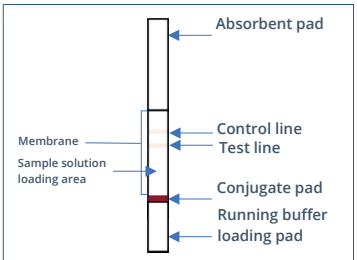

Figure 1: Test Strip architecture

*Optional – You can label the absorbent pad of a **Test Strip** using pencil.*

1. Add **20 µl** of **Running Buffer** to **the final PCR reaction sample**. Mix thoroughly.
2. Add **5 µl** of this solution to the **Test Strip** in the 'Sample solution loading area' of the membrane as marked in Figure 1, above.
3. In a 96-well plate or 1.5 ml tubes, add **300 µl Running Buffer** for each reaction.
4. Place the **Test Strip** in a well or tube containing the reaction and **Running Buffer** mixture.
5. You should leave the test in the buffer solution to run for **10** minutes at which point you can read the test result.

E. Result reading and interpretation

1. A pink solution should flow from the conjugate pad through the membrane and up to the absorbent pad, leaving either a single clear **Control Line** or both **Test and Control Lines**.
2. The test result should be determined after 10

minutes. If the test result is unclear at this point, you can allow the test to run for another 10 minutes when the result can be re-read. You should not read the test more than 1 hour after starting the test run.

3. Occasionally some pink streaks may remain on the membrane. Provided this does not interfere with the reading of the result, the result can be considered valid. If the streaks obscure the test line position, the sample should be re-run.
4. The appearance of the **Control Line** indicates that the reaction/buffer mix has correctly migrated up the **Test Strip** and the test has run correctly. If the pink staining has not run all the way to the absorbent pad, and there are no lines, the result should be considered invalid (see F. Troubleshooting).
5. If only the **Control Line** is visible, the test result is negative. If there is a strong **Test Line** with the negative control sample, the results of all samples run are invalid (see Troubleshooting section below).

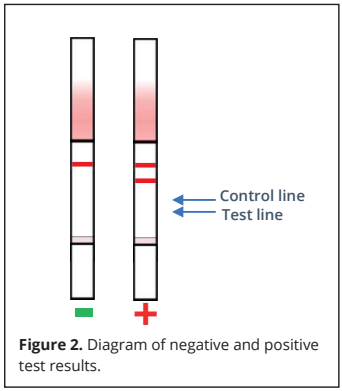

Figure 2. Diagram of negative and positive test results.

6. If both the **Test and Control Lines** are clearly visible, the test result is positive.
7. The **Test and Control Lines** can sometimes vary in intensity. However, if there is mycoplasma present, the **Test Line** will be clear and distinct. The absence of the **Test Line** with just a **Control Line,** indicates there are no mycoplasma species in the sample at detectable levels. If a faint **Test Line** appears, the test should be repeated, either immediately, or after a further 24 hours of culture.

F. Troubleshooting

1. No clear test line with the positive control sample **Test Strip** can be due to the following:
   - I. Thermal cycler programming error
   - II. Pipetting error
   - III. Leaving **Solution I** and **Solution II** mixed before the thermal cycler step for a significant amount of time (>1 hour).
2. A clear test line with a negative control sample can be due to the following:
   - I. Contamination of the control with another mycoplasma-containing sample
   - II. **Test Strip** running error: for example, if the **Test Strip** has been allowed to dry out, i.e., left running for >1 hour
3. No lines appear at all: Check that the **Test Strips** are fully intact and that they have been placed with the correct orientation in the well/tube (absorbent pad **up**).
4. Very faint test lines: Some cell culture media may be more prone to the generation of faint discolouration of the test line. If a sample generates consecutive indeterminate results with at least 24 hours of additional cell culture between tests the sample can be presumed negative. A second test method should be considered for confirmation in this case.
5. Pink staining or smearing of the membrane can occur if the Sample solution is added too low down and flows back into the conjugate pad. If the staining or smearing obscures either line position the test result should be considered invalid.

In any of these cases, samples and controls should be re-run.

G. Disposal

Once a test has been completed, the strip can be discarded with other laboratory waste streams. Whilst the samples have been repeatedly heated, it is good practice to handle the samples as if they retain potential for contamination.

H. Performance

1. Sensitivity: In internal validation studies using a number of different cell cultures, the SwiftDx Mycoplasma Detection Kit was able to correctly identify 100% (66/66, 95% CI: 94.6, 100%) of mammalian cell cultures infected with **10 CFU/ml** of *Mycoplasma hyorhinis* after 3 and 5 days post-contamination.
2. Specificity: In internal validation studies using Day 0 growth samples, known negative samples and negative controls, the SwiftDx Mycoplasma Detection Kit was able to correctly identify 100% (90/90, 95% CI: 96.0, 100%) of mycoplasma negative samples.

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