

MYCOPLASMA GENE DETECTION KIT

P/N 69202; 48 REACTIONS

- Instructions -

Product Features

MycoFinder is a real-time PCR-based assay to detect mycoplasma DNA extracted from mammalian cell cultures. The kit contains PCR strips in which all the reaction reagents are aliquoted and lyophilised to facilitate reagent and sample preparation. MycoFinder is fully compliant with the Japanese, European and US Pharmacopeias (JP 17, EP 2.6.7 and USP 63).

General Precautions

Read the instructions carefully before using the kit. Follow the operational procedures specified in the instructions. Reliability of the kit cannot be guaranteed if the specified operational procedures are not followed or the kit is used for any purposes other than those specified in the instructions. All samples should be considered as infectious, users have to wear appropriate personal protective equipment (labcoats, gloves, safety goggles, face masks).

MycoFinder is intended for research use only. Do not use this kit for other purposes. Do not use in medical diagnostic procedure.

Kit Components

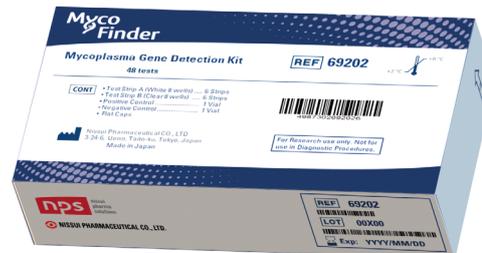
MycoFinder kit contains the following components:

1. 6 x Test strip A (white)*
2. 6 x Test strip B (transparent)*
3. Positive control (2 x 10³ copies /μL)
4. Negative control (DNase Free Water)
5. 6 x Flat caps

*The end-user should select the appropriate PCR strip (white or transparent) with respect to optical properties of the thermocycler to be used.

General Precautions

1. To preserve integrity and performance of the kit; take extra care when handling (use the appropriate personal protective equipment), as the reaction reagents (primers and probes) are sensitive to nuclease.
2. To prevent sample contamination, we recommend sample preparation/ DNA extraction and reagent preparation in two physically distinct work places. If this is not possible, perform UV irradiation or cleaning of surfaces and work space between each step.
3. When taking test strips out of the foil pouch, seal the pouch tightly to preserve the integrity of the remaining test strips and store the MycoFinder kit at +2 to +8°C. Please note that test strips are light sensitive, avoid long exposure to light.
4. Before each use, vortex the positive control and spin it down in a benchtop centrifuge.
5. Use disposable filter tips to avoid contamination of samples during liquid handling.
6. MycoFinder is a real-time PCR-based assay in which amplification and detection are performed simultaneously, there is no need to perform



electrophoresis of PCR products. Do not remove PCR products from test strips as it may cause contamination of your work space.

Operational Procedure

1. DNA extraction method

A number of methods and kits are commercially available to extract DNA from sample; we recommend the end-user to choose carefully a method that is compatible with the cell type and density (1 to 5x 10⁶ cells/ml). The MycoFinder assay is compatible with most extraction methods. For help and advice related to the DNA extraction step, please contact NPS at support@nissui-ps.com.

2. Control preparation

MycoFinder kit contains positive and negative controls. We recommend the end-user to run at least one positive and negative control for each set of experiments.

Positive control: 1 μL of positive control + 24 μL of DNase Free Water.

Negative control: 25 μL of DNase Free Water.

3. How to prepare real-time PCR reaction

25μL of sample to be analysed will be added to a PCR tube in test strip A (white) **OR** B (transparent) and the lyophilised master mix solubilised by pipetting. The solubilised mix is then transferred to a PCR tube in test strip A **OR** B and the master mix solubilisation repeated. The solubilisation sequence (A to B **OR** B to A) is determined by which tube will be used at the end of the process in the PCR instrument. If the thermocycler runs using transparent tubes (B) then start resuspension of Master Mix in Strip A then transfer to Strip B for final resuspension prior to PCR. If the thermocycler runs with white tubes (A) then start resuspension of Master Mix in Strip B then transfer to Strip A for final resuspension prior to PCR.

Procedure

- 3.1- Take the required number of test strips A and test strips B out of the foil pouch.
- 3.2- Add 25μL of extracted DNA to a test strip (A or B – see note above), and pipette gently 20 times to dissolve lyophilised master mix. Liquid has a yellowish colour when fully dissolved.
- 3.3- Transfer all the dissolved sample from the first test strip to the final

test strip and gently pipette 20 times to dissolve the lyophilised reagent.
3.4 Seal the final test strip with the caps, centrifuge strips briefly and load the strips into the real-time PCR instrument.

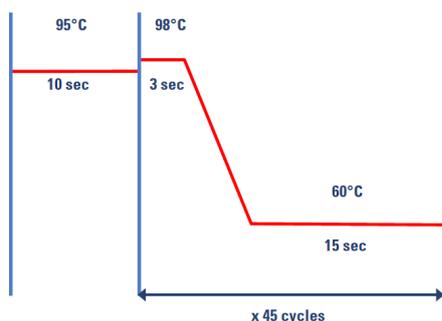
4. Detection by real-time PCR

Nissui Pharmaceutical Solutions has validated the MycoFinder assay on a broad range of thermocyclers. Please refer to the thermocycling technical documentation on our website (www.nissui-ps.com) for optimal detection settings and cycling conditions. The following settings have been optimized for a CFX Connect™ instrument (Biorad).

4.1 Set the fluorescence detection wavelength of the real-time PCR system to FAM™ + ROX™ (or FAM™ + HEX™). Fluorescence signals are as follow:

- a) Mycoplasma detection probe: FAM™
- b) Positive control probe: FAM™ + ROX™ (or FAM™ + HEX™)

4.2 Please refer to the instruction manual of your real-time PCR system to change settings (detection filters and thermal conditions). Recommended cycling conditions for a CFX Connect™ instrument (Biorad) are shown below:



Analysis

We recommend the regression method and background subtracted curve fit mode for analysis. Recommended analysis mode for others thermocyclers can be found on www.nissui-ps.com.

Results Interpretation

1. When FAM™ + ROX™ are set as the detection wavelength, fluorescence intensity of FAM™ will increase for a mycoplasma positive sample. The fluorescence intensity of FAM™ and ROX™ will increase at almost the same cycle quantification value (Cq value) for the positive control. Fluorescence intensity will not increase for the negative control or a negative sample.
2. When FAM™ + HEX™ are set for fluorescence as the detection wavelength, fluorescence intensity of FAM™ will increase for a mycoplasma positive sample. The fluorescence intensity of FAM™ and HEX™ will increase at almost the same cycle quantification value (Cq value) for the positive control. Fluorescence intensity will not increase for the negative control or a negative sample.

Kit Quality Control

The performance of each batch is confirmed by testing 11 standard mycoplasma DNA plasmids at 10 GC/reaction. This covers mycoplasma strains required to be compliant with JP17, EP 2.6.7 and USP 63.

Precautions for Use and Handling

1. Precautions for handling (hazard control)

All samples should be treated as infectious and the end-user must use appropriate personal protective equipment (labcoats, gloves, safety goggles, face masks) and operate according to the best practice guideline for handling hazardous materials.

2. Precautions for use

- 2.1 Do not freeze the product and store under the specified storage condition (+2 to +8°C).
- 2.2 Do not use reagents once expired.
- 2.3 Do not mix reagents from different production batches.

Storage/Expiration Date

Store at +2 to +8°C.
 Expiration date is indicated on the outer package and on the container label.

Limited Product Warranty

This warranty limits our liability for replacement of this product. No warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose are provided. Nissui Pharmaceuticals Co.,Ltd and Nissui Pharma Solutions will have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

Trademarks

CFX9 Connect™ is a trademark of Bio Rad Laboratories, Inc. FAM™, HEX™ and ROX™ are trademarks of Applied Biosystems Corporation or its subsidiaries in the US and certain other countries.

MycFinder kit is manufactured by Nihon Techno Service Co., Ltd. and distributed in Europe by Nissui Pharma Solutions.