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Mycoplasma Gene Detection Kit

Myco Finder™

Instruction Manual

--- Features ---

This product is a kit for detecting mycoplasma genes extracted from cell culture media by real-time PCR. All reaction reagents are provided in solid phase on two types of test strip, so preparation of reagents is easy, and results can be obtained in a short time with simple operation.

--- General Precautions ---

1) Read this instruction manual carefully before use. Use the product in accordance with the operating procedures described in the instruction manual. We do not guarantee the reliability of the product if it is used in a manner other than that described in the operating instructions or for other than the intended purpose of use.
2) All specimens should be treated as potentially infectious and handled with extreme caution while wearing protective equipment (glasses, gloves, masks, etc.).
3) This reagent is for research and should not be used for any other purpose. It is not intended for diagnostic purposes.

--- Kit Composition ---

(1) Test Strip A (white, 8-well)	.....	6
(2) Test Strip B (clear, 8-well)	.....	6
(3) Positive Control (2 x 10^3 copies/μL)	.....	1
(4) Negative Control (DNase Free Water)	.....	1
(5) Flat cap	.....	6

--- Purpose of Use ---

Detection of mycoplasma genes extracted from cell culture media.

--- Operating Precautions ---

1) If probes or primers in the reaction reagent are degraded by nuclease contamination, accurate detection will not be possible. As well as from the experimental equipment, nuclease contamination can come from the sweat and saliva of the user, so take care during operation.
2) To prevent sample contamination, it is recommended that sample preparation and DNA extraction is physically isolated from reaction reagent preparation. If this is difficult, UV irradiation and cleaning of the workspace should be performed before moving on to the next step.
3) Remove the required amount of test strips from the aluminum bag and store the remaining strips with the zipper tightly closed. Since the test strips contain a fluorescent reagent, take care to shield them from light.
4) Test strips should be used with care and not dropped from a high place or subjected to strong impact.
5) The Positive Control should be well mixed and spun down before use.
6) When dispensing reagents, always use new disposable tips to prevent contamination between samples.
7) Operate the real-time PCR device according to its instruction manual.
8) This kit uses a real-time PCR method, and amplification and detection are performed simultaneously, so there is no need to use the amplified product after the reaction is finished for electrophoresis or other purposes. Do not remove the amplified product from the test strip as this may cause contamination.

--- Administration and Dosage ---

[Equipment]

Prepare the following equipment as necessary.

Mixer, micropipette, filter tips (sterilized, DNase/RNase Free), centrifuge, centrifuge tubes, real-time PCR device

[Operating Instructions]

1. DNA extraction

The extraction method using QIAamp® UCP DNA Micro Kit（QIAGEN）is shown below. Other commercially available DNA extraction kits can also be used, but we recommend evaluating the extraction efficiency and other factors beforehand.

1) Add 20 μL of ProteinaseK and 200 μL of Buffer AUL to 200 μL of cell culture medium or centrifugally concentrated cell culture suspension and mix with a mixer.
2) Incubate at 56°C for 15 minutes.
3) Add 200 μL of 99% ethanol and mix with a mixer.
4) Transfer the entire amount to a spin column and centrifuge at 6,000 x g (8,000 rpm) for 1 minute.
5) Transfer the spin column to a new collection tube and add 500 μL of Buffer AUW1. Centrifuge at 6,000 x g (8,000 rpm) for 1 minute.
6) Transfer the spin column to a new collection tube and add 500 μL of Buffer AUW2. Centrifuge at 20,000 x g (14,000 rpm) for 3 minutes.
7) Transfer the spin column to a new collection tube and centrifuge the column empty at 20,000 x g (14,000 rpm) for 1 minute.
8) Make sure there is no liquid residue in the column.
9) Transfer the spin column to a separately prepared centrifuge tube and add 100 μL of Buffer AUE. Incubate at room temperature (15 to 25°C) for 5 minutes.
10) Centrifuge at 6,000 x g (8,000 rpm) for 1 minute and use the resulting sample for reagent preparation.

2. Reagent preparation

1) Remove Test Strips A and Test Strips B from the aluminum bag for the required tests.
2) Add 25 μL of the sample obtained from DNA extraction to Test Strip A and dissolve the solid-phase reagent by gently pipetting 20 times. Dilute the sample accordingly.
3) Transfer the entire dissolved sample volume of Test Strip A to Test Strip B and dissolve the solid-phase reagent by gently pipetting 20 times.
4) Attach a flat cap to Test Strip B and perform detection by real-time PCR.

When detecting with white tubes, add the sample to Test Strip B first and transfer to Test Strip A after dissolution.

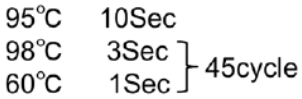
3. Real-time PCR detection

An example protocol for CFX96™ Real-Time PCR Detection System（Bio-Rad） is shown below.

1) Set the fluorescence detection wavelength of the real-time PCR device to FAM + ROX (or FAM + HEX). Note that each fluorescence is used for the following detection.
   (1) Detection of mycoplasma: FAM
   (2) Detection of positive control: FAM + ROX (or FAM + HEX)

   The fluorescence filters must be set to acquire multiple fluorescences before the real-time PCR is performed. When using CFX96™ Real-Time PCR Detection System（Bio-Rad）, the Scan Mode can be set to All channels to read data from all filters installed.
2) The settings of the real-time PCR program should be set based on the

following protocol. For the setting method, follow the instruction manual of the real-time PCR device being used.



--- Determination of Results ---

The results are determined using the analysis software of the real-time PCR device.

CFX96™ Real-Time PCR Detection System（Bio-Rad）recommends analysis using the regression method and baseline subtracted curve fit mode. After analysis, check the amplification curve and confirm that the Cq values are calculated correctly. If the regression method does not analyze correctly due to the purity of the DNA extract, poor mixing of reagents, foaming, etc., use the single threshold method or other methods for analysis. Follow the instructions in the manual provided with the analysis software.

--- Notes for the Determination ---

1) When the fluorescence detection wavelength of the real-time PCR system uses a combination of FAM + ROX, the fluorescence intensity of FAM increases for mycoplasma-positive samples, while for positive controls the fluorescence intensity increases at approximately the same number of cycles (Cq value) for FAM and ROX. For negative controls or negative samples, the fluorescence intensity does not increase.
2) When the fluorescence detection wavelength of the real-time PCR system uses a combination of FAM + HEX, be aware that the fluorescence from FAM may leak into the detection of HEX.

--- Performance ---

1. Sensitivity and accuracy test

When testing this product with plasmid standards (10 copies/test) of *M. arginini*, *M. orale*, *M. salivarium*, *M. hyorhinis*, *M. fermentans*, *M. pneumoniae*, and *Acholeplasma laidlawii* as positive control specimens, and DNase Free Water as a negative control specimen, all positive control specimens will be positive and all negative control specimens will be negative.

2. Product performance data

For detailed performance data, please contact your distributor's sales representative or customer support representative.

3. Additional information on working examples, etc.

Please refer to the following URL for information on working examples, etc.

https://corp.sdc.shimadzu.co.jp/english/products/global/regenerative.html#mycofinder

--- Precautions During Use or Handling ---

1. Handling (hazard prevention)

Specimens should be treated as potentially infectious. To avoid the risk of infection, wear protective equipment (glasses, gloves, masks, etc.) and take care when handling.

2. Use

1) Store at 2 to 8℃ and avoid freezing.
2) Do not use reagents after their expiration date.
3) Do not mix reagents with different lot numbers.
4) Do not use for other purposes.

3. Disposal

1) To avoid contamination, test strips after PCR reaction should be disposed of in a double sealed plastic bag without opening the flat cap as medical waste and according to waste regulations. To prevent PCR amplification products from scattering, do not autoclave.
2) Dispose of used reagents, equipment, etc. as medical waste, industrial waste, or infectious waste in accordance with the provisions of the Act on Waste Management and Public Cleaning and the Water Pollution Prevention Act.

--- Storage and Shelf-life ---

[Storage]

Store at 2 to 8°C.

[Shelf-life]

12 months from date of manufacture

The expiration date is on the label on the outer packaging and container.

--- Packaging ---

Myco Finder™: for 48 tests …… Code69260

--- Contact ---

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