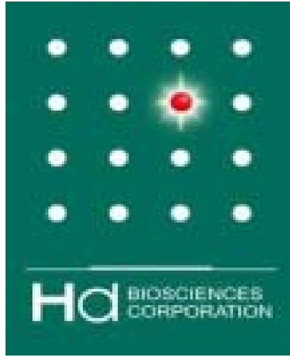

MycoScan™

Mycoplasma Detection Kit for Conventional PCR

INSTRUCTION MANUAL

Catalog #HD01-0105, #HD01-0125,
#HD01-0150, and #HD01-0110

Revision #100302c

For In Vitro Use Only

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ORDERING INFORMATION AND TECHNICAL SERVICES

China

HD Biosciences Co., LTD
Unit 302, 303, 1011 Halei Road,
Shanghai, P.R.C Zip: 201203
Telephone 86-21-58558330
FAX 86-21-58558011
E-mail kit@hdbiosciecnes.com

Other Countries

Please contact your local distributor. A complete list of distributors is available at www.hdbiosciences.com.

MycoScan™ Mycoplasma Detection Kit

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MATERIALS PROVIDED

Instruction manual

1st stage PCR primer mix

2nd stage PCR primer mix
primer sets are lyophilized

Positive Control DNA
DNA-fragments of Mycoplasma genome, prepared by PCR,
non-infectious, lyophilized

Internal Control DNA
Plasmid DNA, non-infectious, lyophilized

STORAGE CONDITIONS

Kit components are stable during shipping. Upon receipt, store at +2oC to +8 oC. After rehydration of the Primer sets, the Positive Control DNA and the Internal Control DNA, store below -18 oC and avoid repeated freezing and thawing.

ADDITIONAL MATERIALS REQUIRED

PCR thermal cycler
mineral oil, if required
PCR tubes¶
agarose gel electrophoresis apparatus
microcentrifuge, micropipettes and filtered tips
deionized, DNA-free water
DNA polymerase

¶ Thin-walled PCR tubes are highly recommended for use. These PCR tubes are optimized to permit more efficient heat transfer and to maximize thermal-cycling performance.

INTRODUTION

MycoScan^{TM} is designed to detect the presence of mycoplasma which might contaminate in biological materials such as cultured cells, utilizing the polymerase chain reaction (PCR) technology. Conventional techniques used to detect mycoplasma involve culturing samples on selective media, which needs at least a week to obtain the results. Whereas, by performing PCR with these primer sets detection results are obtained in a few hours. As the presence of contaminant mycoplasma can be easily detected by only verifying the bands of amplified DNA fragments in electrophoresis, there is no need to prepare probes labeled with radioisotope, etc. This MycoScan^{TM} kit allows detection of species of Mycoplasma, such as *M. fermentans, M. hyorhinis, M. arginini, M. orale, M. hominis, M. arthritidis, M. hyopneumoniae and Acholeplasma laidlawii*, which account for more than 96% of cell culture infections.

TEST PRINCIPLE

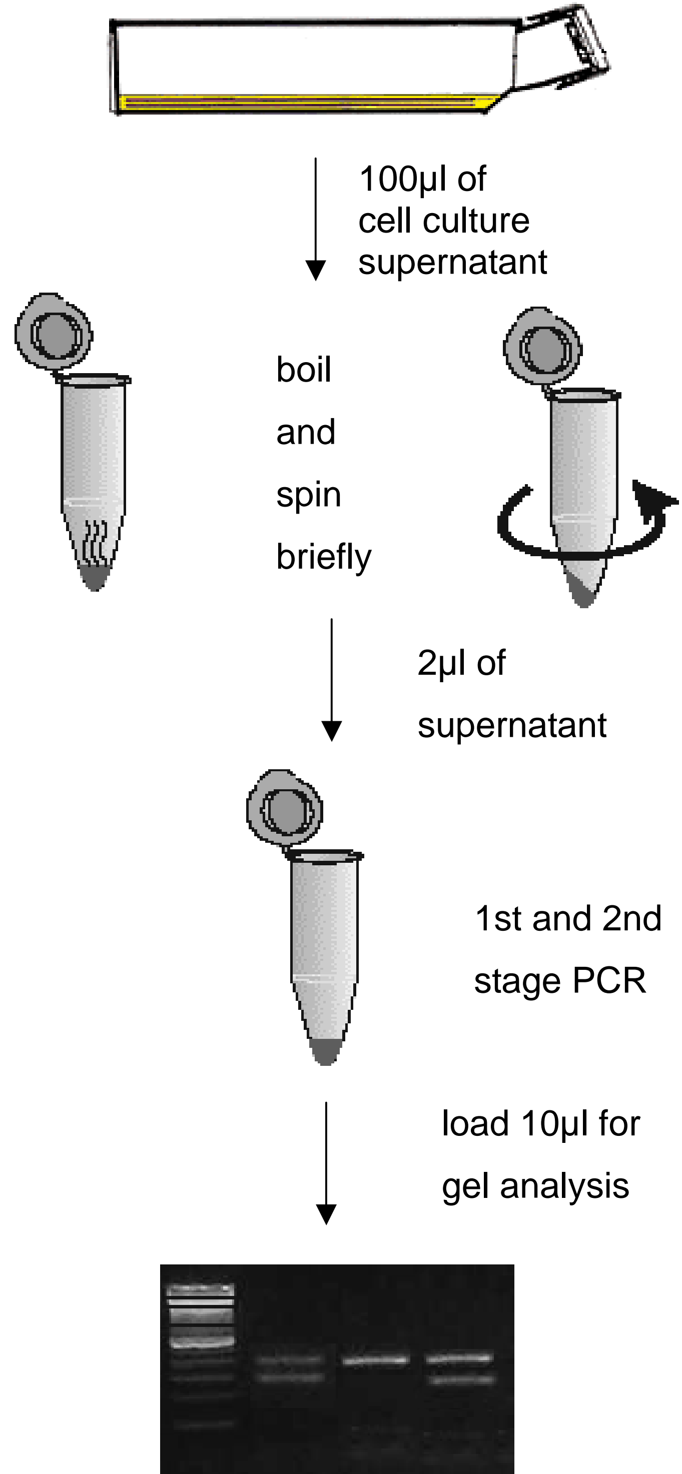
The rRNA gene sequences of prokaryote, including mycoplasma, are well conserved. Whereas, the length and sequences of the spacer region in the rRNA operon (for example, the region between 16S and 23S gene) differs from species to species. Some parts of this spacer region vary depending on species even among mycoplasmas and some parts are well conserved. The detection procedure utilizing PCR process with these primers set is,

1) amplify this spacer region using two primers (F1 and R1) on the DNA encoding rRNA of 16S and 23S
2) perform Nested PCR using two primers F2, which is designed on the basis of this conservative region, and R2 on the 23S gene.

This system does not allow the amplification of DNA originated from other sources, such as cultured cells, which affect the detection result. Amplification of gene sequence with PCR using the MycoScanTM kit enhances not only the sensitivity but also the specificity of detection.

The MycoScanTM also provides internal control DNA, which can be added to the reaction. When running the PCR with the internal control DNA, a successfully performed reaction is indicated by a 498 bp band on the agarose gel.

Scheme of the protocol



PROTOCOL

Sample Preparation

Test samples should be derived from cultures which are at 90-100% confluence. The medium of older cultures may accumulate PCR inhibiting substances. For these sample materials a DNA extraction is strictly recommended prior testing. Penicillin or streptomycin in the culture media do not inhibit mycoplasma or affect test sensitivity. Only cell culture supernatant should be applied to test for mycoplasma. Cell pellets should not be tested, since debris will interfere with the PCR reaction. However, other materials that can be tested are Fetal Calf Serum, vaccines, and paraffin-embedded samples following DNA extraction.

Templates for PCR analysis are prepared by boiling the supernatant of cell cultures or other biologicals for 5 minutes as follows:

1. Transfer 100µl of supernatant from the test culture to a sterile thin-wall PCR tube. The lid should be tightly sealed to prevent opening during heating.
2. Boil or incubate the sample supernatant at 95 oC for 5 minutes.
3. Briefly centrifuge the sample to pellet cellular debris before adding to the PCR mixture.

Rehydration of the Reagents

1. centrifuge tubes with lyophilized components briefly.
2. add appropriate amount of deionized, DNA-free water according to the information provided on the tubes (20 reactions as example):

1st stage and 2nd stage PCR primer mix	50µl/each
Positive control DNA	30µl
Internal control DNA	50µl

3. incubate for 5 minutes at room temperature
4. vortex and centrifuge again

Note: Keep reagents on ice and store below -18 ^{o}C after rehydration.

Frist Stage PCR

1. Prepare the reaction mixture in a thin-wall tube by combining the reagents shown below.

H_2O	29.5 ul
10X PCR buffer	5 ul
dNTPs (2.5mM)	3 ul
1st primer mixture	2 ul
$MgCl_2$ (20mM)	6 ul
Internal control DNA	2 ul
DNA polymerase (5u/ul)	0.5 ul

2. If necessary, overlay mineral oil.
3. Add treated sample 2μl to the above reaction mixture to be 50μl of total volume.
4. Place all tubes in the thermal cycler. Set the parameters by the following condition and perform PCR.

1 cycle	94°C for 2 min
30 cycles	94°C for 30 sec
	55°C for 1 min
	72°C for 1 min
cool down to 4°C	

Notes: the incubation time depends on the polymerase used. Some hot start enzymes need to be activated at 94oC for more than 2 minutes.

Second Stage PCR

1. Prepare the reaction mixture in a thin-wall tube by combining the reagents shown below.

H2O	32.5 ul
10X PCR buffer	5 ul
dNTPs (2.5mM)	3 ul
2nd primer mixture	2 ul
MgCl2 (20mM)	6 ul
DNA polymerase (5u/ul)	0.5 ul

2. If necessary, overlay mineral oil.
3. Add 1st stage PCR products 2µl to the above reaction mixture to be 50µl of total volume.
4. Place all tubes in the thermal cycler. Set the parameters by the following condition and perform PCR.

1 cycle	94°C for 2 min
30 cycles	94°C for 30 sec
	51°C for 30 sec
	72°C for 30 sec
cool down to 4°C	

For controls, add 2µl of DNA template supplied for positive control, and 2µl water for negative control from the 1st stage PCR.

After pipetting the negative control, the tube must be sealed before proceeding with the samples. Also pipetting of the samples and sealing the tubes must be completed before proceeding with the positive control in order to avoid cross contamination.

Agarose Gel Run

- 2% standard agarose gel, approx. 5mm thick, with 5 mm-comb
- load 10µl of each PCR reaction, mixed with bromophenol blue loading buffer per lane
- stop electrophoresis after 2 cm run distance (depending on the electophoresis chamber used)

Gel Evalution

If internal control DNA was used, a distinct 502 bp band should appear in every lane indicating a successfully performed PCR. But this band may fade out with increased amounts of amplicons formed.

Relevant amplicon sizes:

Internal control	502 bp
Mycoplasma sp.	154-237 bp

Your possible band patterns can be interpreted according to following figure:

M 1 2 3 4 5

600

100

FIG. **Possible band patterns.** M, 100 bp DNA marker. 1, negative control. 2, positive control. 3, negative sample. 4, positive sample, weak contamination. 5, positive control, strong contamination.

MycoScanTM designed for high sensitivity and therefore prone to nonspecific annealing, bands of various length that are less intensive can be produced, but do not indicate positive results. Possible primer dimmer produces another band of 89-90 in length, but also does not affect the precision or results of the test.

No amplification of control DNA may be due to the following reasons:

- activity of polymerase is insuffienct
- control DNA tubes have not been spun down before rehydration
- programming mistake
- pipetting mistake

NOTICE TO PURCHASER

This product is optimized for use in the Polymerase Chain Reaction ("PCR") covered by patents owned by Hoffmann-La Roche Inc. and F. Hoffmann-La Roche Ltd ("Roche"). No license under these patents to use the PCR Process is conveyed expressly or by implication to the purchaser by the purchase of this product. A license to use the PCR Process for certain research and development activities accompanies the purchase of certain reagents from licensed suppliers such as LICENSE when used in conjunction with an authorized themal cycler, or is available from Applied Biosystems. Further information on purchasing licenses to practice the PCR Process may be obtained by contacting the Director of Licensing at Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404 or at Roche Molecular Systems, Inc., 1145 Atlantic Avenue, Alameda, California 94501.

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HD BIOSCIENCES Co., LTD

Unit 302, 303, 1011 Halei Road,
Shanghai, P.R.C
Zip: 201203

Tel.: 86-21-58558330
FAX: 86-21-58558011
E-mail: kit@hdbiosciences.com

www.hdbiosciences.com