HiSense™ Mycoplasma PCR Detection Kit (EP)

Version:5.0

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Instruction Manual

Cat. No. HDEP-100

Research Use Only. Not for Use in Diagnostic Procedures.

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1. Product Information

Introduction

HiSense™ Mycoplasma PCR Detection Kit utilizes the PCR, which is established as the method of choice for highest sensitivity in the detection of mycoplasma, ureaplasma and acholeplasma contamination in cell culture and other cell culture derived biologicals. The primers are specific to the highly conserved 16S ribosomal RNA sequences in the mycoplasma genome. This allows for detection of all mycoplasma species including *Acholeplasma laidlawii, M. arginini, M. fermentans, M. gallisepticum, M. genitalium, M. hominis, M. hyorhinis, M. orale, M. pneumoniae, Spiroplasma citri* and *U. urealyticum*. All mycoplasma species including 8 genus and about 209 species can be detected simultaneously. The "European Pharmacopoeia" and Guideline of MFDS recommend checking for unspecific detection of *Clostridium, Lactobacilllus*, and *Streptococcus*. The 16S ribosomal RNA of other bacteria such as *E. coli*, *Clostridium, Lactobacillus, Streptococcus*, and plant and animal cells are not amplified.

Kit Specificity

The HiSense™ Mycoplasma PCR Detection Kit detects Mycoplasma species simply, reliably, and rapidly. To detect the presence of these microorganisms, the assay uses the polymerase chain reaction (PCR) to amplify a target unique to a wide variety of mycoplasmas. The kit can detect more than 209 different Mycoplasma species, including *Acholeplasma laidlawii* and *Spiroplasma citri*. The kit does not detect other genera or cell-line DNA.

Kit Sensitivity

The sensitivity of the PCR using this kit is 1 to 10 copies of the target DNA per reaction. Sensitivity of the assay in real culture samples depends on the quality of the sample preparation.

Prevention of carryover contamination

Take precautions to minimize the potential for carryover of nucleic acids from one experiment to the next. Use separate work areas and pipettes for template preparation and PCR setup steps.

The 2X PCR Master Mix contains dUTP instead of dTTP. When dUTP replaces dTTP in PCR amplification, UNG treatment (Uracil-N-glycosylase, HiSense™ UDG heat labile Cat.No.SCU-100, not provided in this kit) can prevent the subsequent reamplification of dU-containing PCR products. UNG acts on single- and double-stranded dU-containing DNA by hydrolysis of uracil-glycosidic bonds at dU-containing DNA sites. With this strategy, carryover contamination is eliminated, while the template DNA (DNA containing T) remains intact.

Precaution for cross contamination

Cross contamination may occur while handling the positive control DNA contained in the kit. To prevent cross contamination, use separate locations and pipettes for handling positive control during the PCR process. Be sure to use PCR grade water included in the kit and wear gloves during experiments. Additionally, use filter tips to prevent aerosols.

2. Contents and Storage

Materials Provided

Label	Cap	HDEP-100
2X PCR Master Mix	Blue	1.5 ml
Primer Mix	Red	200 µl
Internal Amplification Control DNA	Orange	200 µl
Internal Amplification Control DNA for sample prep	Violet	2 ml
Positive Control DNA	Yellow	50 µl
DNase Free Water	White	1.2 ml

Storage

Upon receipt, store at -20°C.

Note

1) Repeat thawing reduces quality of product.

2) If frequent freeze and thaw is needed, aliquot the products and use in order.

3) Check the label on the product for details.

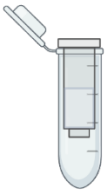
3. Test protocol

Workflow

Sample Preparation

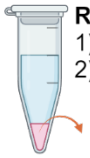
A gDNA Extraction Method

Mycoplasma harvest
1) Transfer 1 ml of cell culture supernatant.
2) Centrifuge (12,000~15,000 rpm, 10min) and discard supernatant.



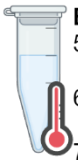
Genomic DNA extraction
3) Follow the DNA extraction kit manual.
add 20µl of Internal Amplification Control DNA (violet cap) at the step of adding Proteinase K
4) 50µl of the elution volume is obtained in the elution step

B Boiling Method



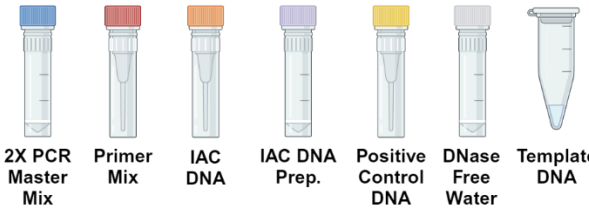
Remove cell debris
1) Transfer 1 ml of cell culture supernatant.
2) Centrifuge (1,000~1,500 rpm, 5min) and transfer 1ml of supernatant to a new tube.

Mycoplasma havest & washing
3) Centrifuge (12,000~15,000 rpm, 10min) and discard supernatant.
4) Add 1 ml D.W or Tris-EDTA buffer and repeat previous step.



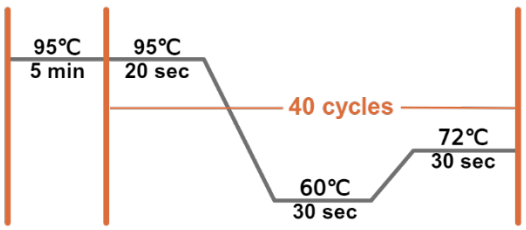
Boiling
5) Discard supernatant, add D.W 100 µl and vortexing.
6) Boiling (98°C,10 min) Centrifuge (12,000~15,000 rpm, 5 min)
7) Transfer 90 µl of supernatant to a new tube.

Reagent Preparation

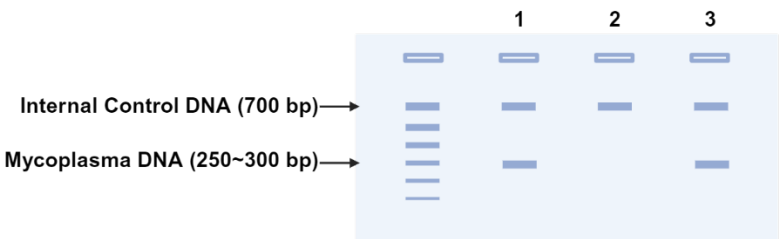


	Method A	Method B	PC	NTC
2X PCR Master Mix	15µl	15µl	15µl	15µl
Primer Mix	2µl	2µl	2µl	2µl
Positive Control DNA	-	-	1µl	-
IAC DNA	-	2µl	2µl	2µl
Template DNA	5µl	5µl	-	-
DNase Free Water	up to 30µl			

PCR Setup and Run



Analyze the results



1. Infected cell
2. No Template Control
3. Positive Control

Prepare the Template (Sample)

Samples should be derived from cultures which are at 90-100% confluence. Penicillin and streptomycin in the culture media do not inhibit PCR or affect test sensitivity. To avoid false positive results, we recommend the use of the PCR grade water delivered with the kit, aerosol-preventive filter tips and gloves.

[A] Genomic DNA Extraction Method

1) Transfer 1 ml of cells with media to a 1.5 ml clean tube. (Use the number of cells according to the recommended amount of the DNA extraction kit.)
2) Centrifuge the tube at 12,000~15,000 rpm for 10 minutes to pellet mycoplasma and discard the supernatant.
3) From the next step, follow the manual for the DNA extraction kit you are using, and add 20 µl of Internal Amplification Control DNA for sample prep (Violet cap) at the step of adding Proteinase K.
4) In the elution step, 50 µl of the elution volume is obtained, and the genomic DNA is used as a PCR template.

[B] Boiling Method

1) Transfer 1 ml of cell culture supernatant to a 1.5 ml clean tube.
2) Centrifuge at 1,000~1,500 rpm for 5 minutes to pellet cell debris, then transfer 1 ml of the supernatant to a clean tube.
3) Centrifuge the tube at 12,000~15,000 rpm for 10 minutes to pellet mycoplasma and discard the supernatant.
   Mycoplasma pellet may not be visible to the naked eye.
4) Add 1 ml of DNase-free water or Tris-EDTA buffer for washing and repeat step 3.
5) Discard the supernatant completely, add 100 µl of DNase-free water into the mycoplasma pellet, and mix by vortexing.
6) Heat the supernatant at 98°C for 10 minutes and centrifuge at 12,000~15,000 rpm for 5 minutes.
   Caution! Be careful when heating the sample at 98°C. Using a PCR machine with a heating cover is recommended.
7) Transfer 90 µl of the supernatant to a fresh tube. Use 5 µl of the supernatant as the template in PCR.

Prepare for PCR

1. **Prepare the set of reactions listed in the following table.**

(Caution!! Don't vigorous vortexing.)

Reaction Components		Sample Reaction	Positive Control Reaction	No Template Control Reaction
Label	Cap			
2X PCR Master Mix	Blue	15 µl	15 µl	15 µl
Primer Mix	Red	2 µl	2 µl	2 µl
Template DNA	-	5 µl	-	-
Internal Amplification Control DNA	Orange	-*	2 µl	2 µl
Positive Control DNA	Yellow	-	1 µl	-
DNase Free Water	White	Up to 30 µl		
Final volume		30 µl	30 µl	30 µl

* When test sample prepared by gDNA extraction method, Internal amplification control DNA is not added separately because the Internal amplification control DNA already included during the sample preparation procedure.

When preparing the sample using the **[B] Boiling Method**, add 2 µl of IAC DNA(Orange cap) in the PCR reaction.

2. **Set up the PCR instrument to run the PCR cycling (amplification) program specified below.**

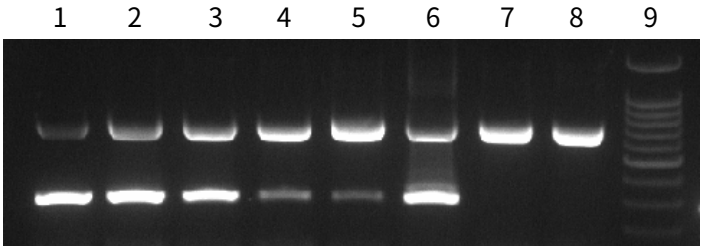
Steps & Cycle			Temp(℃)	Time
Pre-denature			95	5 min
PCR	40 Cycles	Denature	95	20 sec
		Anneal	60	30 sec
		Extend	72	30 sec

3. **Apply 5~10 µl each of PCR products to the gel electrophoresis.**

4. Results / Trouble Shooting

Gel Electrophoresis Result

Tested with Quantitative *M. arginini* Genomic DNA (Cat. No. QAGD, CellSafe)



1. 10^4 copies
2. 10^3 copies
3. 10^2 copies
4. 10^1 copies
5. 1 copy
6. Positive Control
7,8. No Template Control
9. 100bp ladder

When mycoplasma contamination exists, a band with around 250-300bp appears. An internal control DNA band with around 700bp means the right performance of PCR reaction.

Using the following table, determine whether the test cell culture is infected with mycoplasma.

Mycoplasma Band	Internal Control DNA Band	Interpretation
Positive	Positive	Mycoplasma contamination
Positive	Negative*	Mycoplasma contamination
Negative	Positive	Mycoplasma non-contamination
Negative	Negative	PCR inhibition or inadequate sample preparation

* In case of severe mycoplasma contamination, internal control DNA band can be not detected.

1) Recommend performing no template control reaction and positive control reaction by adding 1µl of positive control DNA.
2) If the PCR reaction is inhibited by high FBS concentration, the use of genomic DNA as a template may be helpful.
3) PCR inhibiting substances may accumulate in the medium of cells (e.g., Hybridoma cell, etc.). In this case, the use of diluted sample or genomic DNA as a template may be helpful.