# *UltraSense*
# *Mycoplasma* PCR Detection Kit

## Instruction Manual

**Cat. No. CSHD5(sample), CDHD25, CSHD50 and CSHD100**

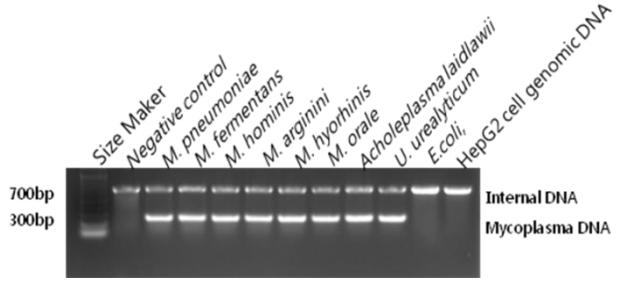
**Research Use Only. Not for Use in Diagnostic Procedures.**

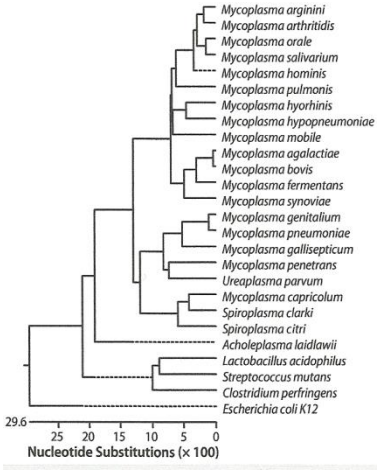
# *UltraSense* PCR Detection Kit

## Introduction

*UltraSense* 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 KFDA recommend checking for unspecific detection of Clostridium, Lactobacilllus, and Streptococcus. The 16S ribosomal RNA of other bacteria such as *E.coli, Clostridium, Lactobacillus, Sterptococcus*, and plant and animal cells is not amplified.

.




## Kit Specificity

The *UltraSense* 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 less than 10fg of the target DNA per reaction. Sensitivity of the assay in real culture samples depends on the quality of the sample preparation.

## Preventing Template Cross-Contamination

Take precautions to minimize the potential for carryover of nucleic acids from one experiment to the next. Use separate work areas and pipettors for template preparation and PCR setup steps. Use the PCR grade water delivered with the kit, aerosol-preventive filter tips and gloves.

The 2×QPCR master mix contains dUTP instead of dTTP. When dUTP replaces dTTP in PCR amplification, UNG treatment (Uracil-N-glycosylase, 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. When this strategy is put to use, carryover contamination will be eliminated while template DNA (DNA containing T) will be left intact.

## Materials Provided

| Materials Provided | Quantity | | | |
|---|---|---|---|---|
| | **CSHD5 (Sample)** | **CSHD25** | **CSHD50** | **CSHD100** |
| 2x PCR Master Mix (Blue Cap) | 75μℓ | 375μℓ | 750μℓ | 1.5ml |
| Primer Mix (Red Cap) | 10μℓ | 50μℓ | 100μℓ | 200μℓ |
| Positive Control DNA (Yellow Cap) | 3μℓ | 13μℓ | 25μℓ | 50μℓ |
| DNase Free Water (White Cap) | 60μℓ | 300μℓ | 600μℓ | 1.2ml |

## Storage Conditions

*Upon receipt, store at -20 ℃. Full activity is guaranteed for 12 months.*

## Test Protocol

## 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 mycoplasma 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.

1. **High contamination : Heat-inactivation of the sample material**

   The templates for the PCR analysis are prepared by direct heating of the samples (the cell culture supernatant or the biological material).

   1) 150μℓ liquid supernatant of the sample is transferred into a sterile reaction tube.
   2) The supernatant is centrifuged (5 minutes, 1,000 x g) to sediment cell debris.
   3) 100ul of the supernatant is transferred into a sterile reaction tube.
   4) The supernatant is incubated at 95℃ for 10 minutes
   5) The supernatant is used as template in the PCR. If the template contains PCR inhibition materials, the DNA can be purified with a commercial extraction kit.

2. **Low contamination : Enrichment of mycoplasma by centrifugation**

   1) 1.2 ml liquid supernatant of the sample is transferred into a sterile 1.5ml reaction tube and centrifuged (5 minutes, 1,000 x g) to sediment cell debris.
   2) 1ml of the supernatant is transferred into a sterile 1.5ml reaction tube.
   3) The supernatant is centrifuged (10 minutes, 13.000 x g) to sediment mycoplasma particles.
   4) The supernatant is rejected and the pellet is suspended into 50ul buffer (10 mM Tris, pH 8.0).
   5) The suspended pellet should be vortexed and finally heated up to 95℃ for 10 min.
   6) The heated suspension can be stored at -20℃ for a period of one year. Repeated freezing and defrosting, or storage in the refrigerator for longer than 12 hours should be avoided.
   7) If the template contains PCR inhibition materials, the DNA can be purified with a commercial extraction kit.

3. **Genomic DNA extraction**

   1) Prepare $5x10^6 \sim 1x\ 10^7$ cells in 10ml culture
   2) DNA was isolated using a commercial kit, QIAamp® DNA Mini (Qiagen, Valencia, CA) or equivalent products of it following the procedure provided by the vendor.
   3) The concentration of genomic DNA was determined by UV260 measurement.
   4) Take 5μL in final 50μL DNA solution as template for PCR reaction.

**Prepare for PCR**

1. Prepare the set of reactions listed in the following table. These include two types of control reactions: 1) **positive control** reaction(s) containing Mycoplasma positive control template DNA, and 2) a **negative control** (no template).

| Reaction Components | Sample Reaction (Sample, $\mu l$) | Control Reactions | |
|---|---|---|---|
| | | Positive Control (Control DNA) | Negative Control (Water) |
| 2X Master Mix | 15 | 15 | 15 |
| Primer mix | 2 | 2 | 2 |
| Template | 1~10 | - | - |
| Control DNA | - | 1 | - |
| $H_2O$ (up to 30) | | | |
| Final volume | 30 | 30 | 30 |

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

| Steps | | | Temp(℃) | Time |
|---|---|---|---|---|
| Pre Heat | | | 94 | 5 min |
| PCR | 40 Cycles | Denature | 94 | 20 sec |
| | | Anneal | 60 | 30 sec |
| | | Extend | 72 | 30 sec |

3. Apply 10~20 μl each of PCR products to the gel electrophoresis.

## Results

1. When mycoplasma contamination exists, a band with around 250-270bp appears. An internal DNA band with around 700bp means the right performance of PCR reaction.

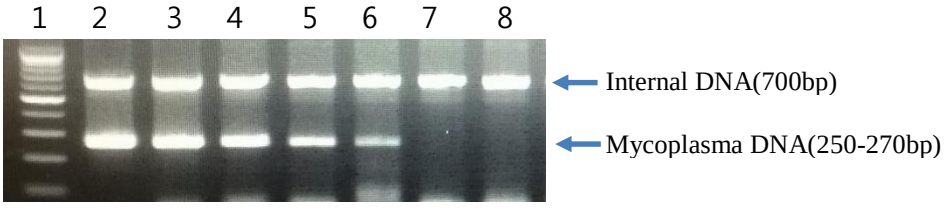



Fig. 1. Lane 1, 100bp DNA ladder; lane 2, 10pg *M. pneumoniae* DNA; lane 3, 1pg *M. pneumoniae* DNA; lane 4, 100fg *M. pneumoniae* DNA; lane 5, 10fg *M. pneumoniae* DNA; lane 6, 1fg *M. pneumoniae* DNA lane 7, 8, PCR reagent control (negative control)

Note:
1) Recommend to perform one negative control without sample and one positive control reaction by adding 1μl of mycoplasma 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 hybridoma cell. In this case, the use of diluted sample or genomic DNA as a template may be helpful.

**Mycoplasma species detected by *UltraSense* Mycoplasma PCR Detection Kit**

***A.** **Acholeplasma laidlawii***
***B.** **Mycoplasma arginini***
***C.** **Mycoplasma fermentans***
***D.** **Mycoplasma gallisepticum***
***E.** **Mycoplasma hyorhinis***
***F.** **Mycoplasma orale***
***G.** **Mycoplasma pneumoniae***
***H.** **Mycoplasma synoviae***
***I.** **Spiroplasma citiri***
*J. Mycoplasma agalactica*
*K. Mycoplasma alligatoris*
*L. Mycoplasma anatis*
*M. Mycoplasma arthritidis.*
*N. Mycoplasma bovis*
*O. Mycoplasma bovigenitalium*
*P. Mycoplasma capricolum*
*Q. Mycoplasma cloacale*
*R. Mycoplasma falconis*
*S. Mycoplasma faucium*
*T. Mycoplasma flocculare*
*U. Mycoplasma gallinarum*
*V. Mycoplasma genitalium*
*W. Mycoplasma hominis*
*X. Mycoplasma pneumoniae*
*Y. Mycoplasma hyopharyngis*
*Z. Mycoplasma hyosynoviae*

27. *Mycoplasma imitans*
28. *Mycoplasma maculosum*
29. *Mycoplasma meleagridis*
30. *Mycoplasma mycoides*
31. *Mycoplasma muris*
32. *Mycoplasma neurolyticum*
33. *Mycoplasma opalescens*
34. *Mycoplasma penetrans*
35. *Mycoplasma pirum*
36. *Mycoplasma primatum*
37. *Mycoplasma pulmonis*
38. *Mycoplasma putrefaciens*
39. *Mycoplasma salivarium strain*
40. *Mycoplasma spermatophilum*
41. *Mycoplasma sp. ovine/caprine*
42. *Mycoplasma sp. putative*
43. *Mycoplasma suis*
44. *Mycoplasma sualvi*
45. *Mycoplasma timone*
46. *Ureaplasma diversum*
47. *Ureaplasma urealyticum*

**Mycoplasma species in KFDA guidance on the Mycoplasma Test*