Cat. # 6601

For Research Use

TaKaRa

TaKaRa PCR Mycoplasma Detection Set

Product Manual

v201808Da

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I. Description

TaKaRa PCR Mycoplasma Detection Set is a primer set designed to detect the presence of *Mycoplasma* which might contaminate biological materials such as cultured cells. The assay using this product can detect *Mycoplasma* within a few hours by PCR and electrophoresis, unlike the cell culture assay, which requires about one week.
This primer set allows sensitive and specific detection of several different species of *Mycoplasma,* (*M. fermentans, M. hyorhinis, M. arginini, M. orale, M. salivarium, M. hominis, M. pulmonis, M. arthritidis, M. neurolyticum, M. hyopneumoniae, M. capricolum*) and one species of *Ureaplasma* (*U. urealyticum*). This primer set will work most efficiently when used in conjunction with *TaKaRa Taq*™ or *TaKaRa Ex Taq*®.

II. Components (100 reactions x 50 μl PCR)

1. MCGp F1 Primer (20 pmol/μl) 50 μl
2. MCGp R1 Primer (20 pmol/μl) 50 μl
3. MCGp F2 Primer (20 pmol/μl) 50 μl
4. MCGp R2 Primer (20 pmol/μl) 50 μl
5. Control Template (1 ng/μl) 50 μl

III. Storage -20℃

IV. Materials Required but not Provided

[Reagents]

1. *TaKaRa Taq* (Cat. #R001A/B/C) or *TaKaRa Ex Taq* (Cat. #RR001A/B/C)
2. Agarose gel
   ex. Agarose L03 [TAKARA] (Cat. #5003/B)
   PrimeGel™ Agarose PCR-Sieve (Cat. #5810A)
3. Sterile purified water

[Equipment]

1. Authorized instruments for PCR
2. Microcentrifuge tubes (made of polypropylene)
3. Agarose gel electrophoresis apparatus
4. Microcentrifuge
5. Micropipets and pipette tips (autoclaved)

V. Principle

The rRNA gene sequences of procaryotes, including *Mycoplasma*, are well conserved. Whereas, the lengths and sequences of the spacer regions in the rRNA operon (for example, the region between the 16S and 23S genes), differ from species to species. Some parts of this spacer region vary even among *Mycoplasma* species, while others are well conserved. This primer set is used in a PCR detection assay to:

1) amplify this spacer region using two primers (F1 and R1), which were designed based on the DNA encoding the 16S and 23S rRNAs.
2) perform Nested PCR using two primers, F2, based on the conserved region, and R2, based on the 23S gene (see Fig 1).

This system does not allow the amplification of DNA from other sources, such as cultured cells, which affect the detection results. PCR amplification of gene-specific sequences using this primer set enhances both the sensitivity and specificity of detection. Table 1 shows the sequences of the four primers provided in this set and the respective sequences in the 16S-spacer-23S region of 12 species of *Mycoplasma* which correspond to these four primers.

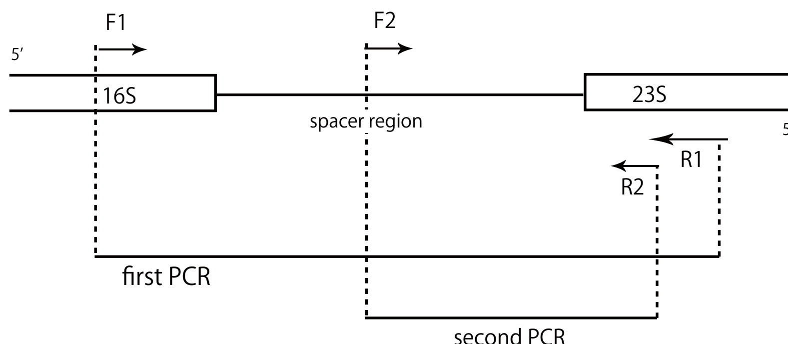


Figure 1. Principle of PCR-based *Mycoplasma* detection

	F1	R1	F2	R2
	5'ACACCATGGGAGCTGGTAAT3'	5'CTTCATCGACTTTCAGA-CCCAAGGCAT3' T (under A, position 5); C (under T, position 13)	5'GTTCTTTGAAAACTGAAT3'	5'GCATCCACCAAAAACTCT3' T (under A, position 11); T (under A, position 13)
M. fermentans	••••••••••••••••••••	••••A•••••••T••••-••••••••••	••••••••••••••••••	••••••••••C•A•••••
M. hyorhinis	••••••••••••T•••••••	••••A•••••••T••••-••••••••••	••••••••••••••••••	••••••••••A•T•••••
M. orale	••••••••••••••••••••	••••T•••••••T••••-••••••••••	A•••••••••••••••••	••••••••••A•A•••••
M. hominis	••••••••••••••••••••	••••T•••••••T••••-••••••••••	••••••••••••••••••	••••••••••A•A•••••
M. salivarium	••••••••••••••••••••	••••T•••••••T••••-••••••••••	••••••••••••••••••	••••••••••A•A•••••
M. arginini	••••••••••••••••••••	••••A•••••••C••••-••••••••••	••••••••••••••••••	••••••••••A•A•••••
M. capricolum	••••••••A•••T•••••••	••••T•••G••-C••TTTT•••••••••	••••••••••••••••••	••••T•••••T•TG••••
M. arthritidis	••••••••••••••••••••	••••A•••••••C••••-••••••••••	••••••••••••••••••	••••••••••A•A•••••
M. neurolyticum	••••••••••••T•••••••	••••A•••••••C••••-••••••••••	A•••••••••••••••••	••••••••T•T•T•••••
M. pulmonis	••••••••••••••••••••	••••T•••••••C••••-••••••••••	•••••••A••••••••••	••••••••T•C•A•••••
M. hyopneumoniae	••••••••••••T•••••••	•C••A•••••••T••••-••••••••••	•C•••••C••••••••••	••••••••••A•T•••••
U. urealyticum	•A••T•••••••••••••••	••••A•••C••GTTT•T-••••••••••	•A••••••••••••••••	••••T•••••T•AG••••
E. coli	••••••••••••TG•••TGC	••••A•••C••CTG•CT-G•••G•••••	------------------	•••••••••GTGT••G••
B. subtilis	••••••C•A•••T•T••••C	••••A•••G••CCT••T-G•••••••••	••••••••••••••AG••	•••••••••GTGCG•C••

Table 1. Sequences of primers and their corresponding *Mycoplasma* DNA
・Contains the same nucleotide as the primers.
- Does not contain the same nucleotide as the primers.

VI. Considerations Before Use

This kit is designed to detect *Mycoplasma* DNA and can also detect non-viable bacteria. *Mycoplasma* DNA cannot be detected in some cases, when a mutation or deletion/insertion occurs within the sequence covered by the primers in the kit.
(Takara Bio is not responsible for any actions taken as a result of analytical determinations made with this product.)

VII. Precautions

The nested PCR reaction used in this kit is a very sensitive amplification method.
Take care to avoid cross-contamination with PCR products and the Control Template. It is recommended to designate and physically segregate work areas for reaction mixture preparation through detection.

VIII. Protocol

It is recommended to use cell culture supernatants incubated for 3 - 6 days after subculturing as samples for *Mycoplasma* detection. If the cells are grown in a medium such as Eagle Medium, the supernatant can be added directly to the PCR reaction. If cell suspension samples are used, extract the DNA and add it to the PCR reaction.
In cases where PCR inhibitors might be present in the sample, it is recommended to extract the DNA from the sample before adding it to the PCR reaction. In order to confirm that a sample does not contain any PCR inhibitors, a positive control experiment using the Control Template should be performed. (Refer to D. Control Template experiment on page 7.)

A. 1st PCR

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

Reagents	Volume
Sterile purified water	34.75 - 39.25 μl
10X PCR Buffer	5 μl
dNTP Mixture	4 μl
MCGp F1 Primer	0.5 μl
MCGp R1 Primer	0.5 μl
TaKaRa Taq or *TaKaRa Ex Taq*	0.25 μl

2. Add sample (less than 5 μl) to the above reaction mixture to bring the total volume up to 50 μl.
   Note: Care should be taken to prevent cross contamination when adding sample.

3. Place all tubes in a thermal cycler and perform PCR under the following conditions.

   94℃ 30 sec 1 cycle
   ↓
   94℃ 30 sec
   55℃ 2 min
   72℃ 1 min
   30 - 35 cycles

B. 2nd PCR

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

Reagents	Volume
Sterile purified water	39.25 μl
10X PCR Buffer	5 μl
dNTP Mixture	4 μl
MCGp F2 Primer	0.5 μl
MCGp R2 Primer	0.5 μl
TaKaRa Taq or *TaKaRa Ex Taq*	0.25 μl

2. Carefully add 0.5 μl* of the 1st PCR product to the above reaction mixture.
   Note: Care should be taken to prevent cross-contamination when adding the 1st PCR products.
   * To obtain clear results, also prepare reactions containing 10-fold- or 100-fold-diluted 1st PCR products.

3. Place all tubes in a thermal cycler and perform PCR under the following conditions.

94℃	30 sec	1 cycle
↓		
94℃	30 sec	30 cycles
55℃	2 min	
72℃	1 min	

C. Analysis of amplified products by gel electrophoresis

1. Set aside 10 μl each of the 1st and 2nd PCR products for analysis using gel electrophoresis.

2. Perform agarose gel electrophoresis of the PCR products to verify the presence of the amplified product and its size. For example, a 1% agarose gel (e.g. Agarose L03 [TAKARA] (Cat. #5003/B)) was used for analysis of 1st PCR products and a 2 - 4% PrimeGel Agarose PCR-Sieve (Cat. #5810A) was used for analysis of 2nd PCR products.
   * Table 2 shows the sizes of DNA fragments amplified using two different primer pairs when 12 species of *Mycoplasma* DNA are used as templates.

Table 2. Sizes of amplified DNA fragments of 12 species of *Mycoplasma*

Species	Primer Pairs	
	F1 and R1 (bp)	F2 and R2 (bp)
M. hyopneumoniae	681	237
M. neurolyticum	501	196
M. fermentans	491	195
M. pulmonis	477	189
M. hyorhinis	448	211
M. orale	423	179
M. capricolum	415	179
M. arthritidis	408	157
M. salivarium	403	151
M. hominis	370, 369	147, 148
M. arginini	369	145
U. urealyticum	482, 481	154

D. Control Template experiment

In order to confirm that a sample does not contain any inhibitors, a positive control experiment using the Control Template should be performed. In this control experiment, the sample (or the cell culture medium) should be added to the PCR reaction together with 0.5 μl of the supplied Control Template.
If the sample does not contain any inhibitors, the bands derived from the Control Template will be visible. (An 810-bp band will appear with the F1 and R1 primers, and a 590-bp band will appear with the F2 and R2 primers.)

Note:

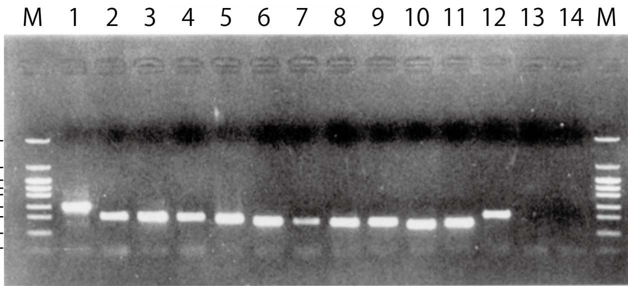
1. If the bands derived from the Control Template are not visible, this may be due to the presence of a PCR inhibitor. In this case, it is recommended to extract DNA from the sample before using it as a PCR template.
2. The bands derived from *Mycoplasma* may not be detected by adding the Control Template to the PCR reaction, even if the sample contains *Mycoplasma*. Therefore, the positive control experiment cannot be used for *Mycoplasma* detection. For accurate *Mycoplasma* detection, a PCR reaction using only sample as a template should be performed.
3. When the sample used for the positive control experiment contains a large amount of *Mycoplasma*, the bands derived from the Control Template may not be detected, although the bands from *Mycoplasma* can be detected.
4. The Control Template does not contain any *Mycoplasma* sequence.

IX. Application
(Detection results with 11 species of *Mycoplasma* and 1 species of *Ureaplasma* using PCR)

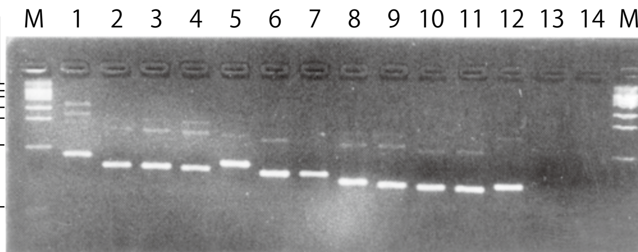
DNA was extracted from cultured *Mycoplasma* (11 species) and *Ureaplasma* (1 species). Nested PCR was performed in 100 μl PCR using extracted DNA (~1 ng), or human DNA and mouse DNA as a negative control.

[Result]

1st PCR



2nd PCR



Lane M : pHY Marker
1 : *M. hyopneumoniae*
2 : *M. neurolyticum*
3 : *M. fermentans*
4 : *M. pulmonis*
5 : *M. hyorhinis*
6 : *M. orale*
7 : *M. capricolum*
8 : *M. arthritidis*
9 : *M. salivarium*
10 : *M. hominis*
11 : *M. arginini*
12 : *U. urealyticum*
13 : human DNA
14 : mouse DNA

In the 1st PCR, the detection limits are 1 ng for *M. capricolum,* 100 pg for *M. hyopneumoniae* and *U. urealyticum*, and 10 pg for other *Mycoplasma* species. (Non-specific bands were detected when 100 ng of human or mouse DNA was used as a template.)
In the 2nd PCR, the detection limits are 100 fg for *M. capricolum* and *M. hyopneumoniae,* and 10 fg for other *Mycoplasma* species. (Nonspecific bands were not detected when using 100 ng of human or mouse DNA as a template.)

X. Q & A

Q1: What medium is available for preparing cell culture supernatant samples that can be analyzed using this product?

A1: The following medium was used to prepare samples for *Mycoplasma* detection reactions with *TaKaRa Ex Taq* and *TaKaRa Taq*:

(1) The supernatant of a cell culture grown for 3 - 6 days in DMEM and RPMI medium (including 10% FCS after subculturing) was added directly to a PCR reaction in one-tenth the reaction volume. The amplification of *Mycoplasma* DNA was confirmed.
(2) It was confirmed that the PCR reaction would not be inhibited by adding FCS in one-tenth volume.

Q2: Can this kit be used for detection of *M. pneumoniae*?

A2: No.

XI. Reference

Uemori, T., Asada, K., Kato, I., and Harasawa, R. *System. Appl. Microbiol*. (1992) **15:**181-186.

X. Related Products

TaKaRa Taq™ (Cat. #R001A/B/C)
TaKaRa Taq™ Hot Start Version (Cat. #R007A/B)
TaKaRa Ex Taq® (Cat. #RR001A/B/C)
TaKaRa Ex Taq® Hot Start Version (Cat. #RR006A/B)
CycleavePCR™ Mycoplasma Detection Kit (Cat. #CY232)*

* Not available in all geographic locations. Check for availability in your area.

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