

Mycoplasma sp. PCR detection Kit

Cat. No.: PK3131
Store at: -20°C

Shipment: Dry or gel ice
Quantity: 20 Reactions

Description: This kit is destined for qualitative detection of Mycoplasma DNA by the method of Polymerase Chain Reaction. The SinaClon *Mycoplasma sp.* **PCR detection Kit** offers convenient reagent for PCR amplifications and detection of Mycoplasma contamination in cell culture and other cell culture derived biologic materials. The reagent of ready to use mix is an optimized 1X PCR mixture of Taq DNA Polymerase (recombinant), PCR buffer, MgCl₂, dNTPs and primers. Primer set is specific to the highly conserved 16srRNA coding region in the Mycoplasma genome. This primer set, allows for detection of about 50 species of Mycoplasma¹. Detection requires at least 1-5 fg Mycoplasma DNA or 2-5 Mycoplasma per sample. The mix contains all components for PCR expect DNA. Additionally, sterile water, PCR grade mineral oil and Positive control has supplied. Positive control tube contains a plasmid with cloned PCR fragment which indicates a successful performed reaction. Blue and ready to load mix does not need to any loading dye for electrophoresis. 280 bp PCR products indicate a positive reaction.

This Kit is sufficient for 20 amplification reactions of 25 µl volume each.

Kit Contents:

1. 1X PCR MIX	400 µl
2. Taq DNA polymerase (5u/µl)	5.0 µl
3. Mineral Oil	1.0 ml
4. Positive Control	50 µl
5. DNase Free, Deionized Steril Water	1.0 ml

• The Kit should be stored at -20°C.

Cycling parameters*:

Initial Denaturation:	94°C - 2 min	1Cycle
	94°C - 15 sec.	} 35 Cycles
	52°C - 20 sec.	
	72°C - 35 sec.	
Final Extension:	72°C - 5 min	1Cycle

*(Cycling parameters may need to be setup with some Thermocyclers)

DNA Extraction

Performed in Pre-amplification 1 , Specimen & Control Area.

1. For high contaminated cell culture, supernatant can be use: Transfer 100µl of supernatant to 1.5ml tube; incubate at 95°C for 5 minutes. Pellet cellular debris by short centrifuge for 5 seconds. Use 5µl supernatant as a PCR template.
2. For suspected or older cultures and other cell culture derived biologic materials DNA extraction should be performed. Sample DNA can be extracted using **SinaClon DNP™** (Cat.No.:EX6071) or **SinaPure™Gram Negative DNA Extraction Kit** (Cat.No.:EX6011).

PCR Protocol

Performed in Pre- Amplification 2 , Reagent Preparation Area.

1. Take out the kit and unfreeze the tubes, then put all the tubes on ice. The final volume of each reaction will be 25µl.
2. Label new PCR tubes for amplification reaction(s) for test(s), positive and negative control.
3. Add the following reagents for each tube on ice:
PCR MIX 20 µl/ for each reaction
Taq DNA polymerase 0.2µl/ for each reaction

4. To each tube add one drop (20-25 µl) mineral oil (if needed). Cap the reactions tube or Place the tube tray in a resalable plastic bag and seal the bag securely.

Next steps should be done at:

Pre –Amplification 1 , Specimen & Control Preparation Area

5. Add 5 µl DNA*(Use specified pipette for sampling of DNA).
6. Close tubes; spin the mixtures on microfuge for 3-5 sec.
7. Transfer the tubes to preheated thermocycler and start the program:

Results Analysis

Performed in Post –Amplification Area.

Analyze 10 µl of amplified samples directly in a 1.5-2% agarose gel without adding loading buffer. The presence of **280 bp** fragments indicates **positive test**.

Mycoplasma species can be detected by SinaClon Mycoplasma detection kit:

1	<i>Mycoplasma pneumoniae</i>	17	<i>Mycoplasma glycophilum</i>	33	<i>Mycoplasma testudineum</i>
2	<i>Mycoplasma orale</i>	18	<i>Mycoplasma synoviae</i>	34	<i>Mycoplasma timone</i>
3	<i>Mycoplasma hyorhinis</i>	19	<i>Mycoplasma iguanae</i>	35	<i>Mycoplasma zalophi</i>
4	<i>Mycoplasma arginini</i>	20	<i>Mycoplasma lagogenitalium</i>	36	<i>Mycoplasma verecundum</i>
5	<i>Mycoplasma salivarium</i>	21	<i>Mycoplasma lipofaciens</i>	37	<i>Mycoplasma citelli</i>
6	<i>Mycoplasma hominis</i>	22	<i>Mycoplasma moatsii</i>	38	<i>Mycoplasma alligatoris</i>
7	<i>Mycoplasma fermentas</i>	23	<i>Mycoplasma molare</i>	39	<i>Mycoplasma anatis</i>
8	<i>Mycoplasma agalactiae</i>	24	<i>Mycoplasma mustelae</i>	40	<i>Mycoplasma buteonis</i>
9	<i>Mycoplasma bovis</i>	25	<i>Mycoplasma opalescens</i>	41	<i>Mycoplasma canis</i>
10	<i>Mycoplasma vulturii</i>	26	<i>Mycoplasma oxoniensis</i>	42	<i>Mycoplasma sphenisci</i>
11	<i>Mycoplasma bovis genitalium</i>	27	<i>Mycoplasma phocicerebrale</i>	43	<i>Mycoplasma collis</i>
12	<i>Mycoplasma conjunctivae</i>	28	<i>Mycoplasma phocidae</i>	44	<i>Mycoplasma crocodyli</i>
13	<i>Mycoplasma dispar</i>	29	<i>Mycoplasma spumans</i>	45	<i>Mycoplasma phocae</i>
14	<i>Mycoplasma gallopavonis</i>	30	<i>Mycoplasma sualvi</i>	46	<i>Mycoplasma cynos</i>
15	<i>Mycoplasma hyosynoviae</i>	31	<i>Mycoplasma columborale</i>	47	<i>Mycoplasma sp.</i>
16	<i>Mycoplasma orale strain</i>	32	<i>Mycoplasma cricetuli</i>		

For Research Use Only

SinaClonBioScience

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