

ab289834 – Mycoplasma PCR Detection Kit

PCR based kit to detect mycoplasma in cell culture media. For research use only - not intended for diagnostic use.

Storage and Stability

Upon arrival, the kit should be stored at -20°C. Avoid repeated freeze-thaw cycles of the MasterMix to retain maximum performance.

Materials supplied

Item	Quantity	Storage condition
ExpressTaq 2X PCR MasterMix	1.25 mL	-20°C
Mycoplasma PCR Primer Mix	100 µL	-20°C
Mycoplasma Positive Control	250 µL	-20°C
Nuclease-free water	1 mL	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Thermal Cycler
- PCR tubes
- Nuclease-free water
- 1.5 ml microcentrifuge tubes
- Reagents and equipment for agarose gel electrophoresis
- Ethidium bromide or Safe Image™ Basic DNA Stain
- DNA ladder

PCR Detection Protocol

1. Collected samples can be stored at 4°C for 3-5 days before testing. The cells should remain in culture for at least 48-72 hours undisturbed prior to screening for the presence of mycoplasmas and should be at least 80% confluent. Alternatively, the supernatant can be frozen and thawed before testing provided that the supernatant was in culture 48-72 hours before testing. Collect 2.5 µL directly from culture.
2. Mix individual components before use and assemble reaction on ice.

Components	Test Samples	Positive Control	Negative Control
ExpressTaq 2X PCR MasterMix	12.5 µL	12.5 µL	12.5 µL
Mycoplasma PCR Primer Mix	1 µL	1 µL	1 µL
Test sample	2.5 µL	-	-
Mycoplasma Positive Control	-	2.5 µL	-
Nuclease-free water	9 µL	9 µL	11.5 µL
Final volume per reaction	25 µL	25 µL	25 µL

3. Gently mix the reaction and briefly centrifuge. Run thermocycling conditions for standard PCR.
4. Perform 30-40 cycles of PCR as follows:

Step	Temperature	Duration	Cycle(s)
Enzyme Activation	95°C	3 min	1
Denaturation	95°C	15 sec	30-40
Annealing	55°C	15 sec	30-40
Extension	72°C	15 sec	30-40
Final extension	72°C	1 min	1
Holding	4°C	-	-

5. After PCR, maintain the reaction at 4 °C or store at -20 °C until use.
6. Analyze the amplification products by agarose gel electrophoresis.
7. Visualize by Safe Image™ Basic DNA Stain or ethidium bromide staining.
8. The presence of PCR product of ~500 bp in length indicates that the cell culture tested is contaminated with mycoplasma. Note that the length of the PCR product will vary between 370-550 bp depending on different mycoplasma species/strains.

Recommendations for Optimal Results:

Always keep the reaction mixture chilled on ice prior to running the PCR and start the PCR as soon as the reaction mixture is prepared.

Technical Support

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Appendix

List of Species/Strains Detected:

No.	Mycoplasma Species/Strains	No.	Mycoplasma Species/Strains
1	Mycoplasma arginini strain EF-Hungary	19	Mycoplasma mustelae
2	Mycoplasma auris	20	Mycoplasma mustelae strain MX
3	Mycoplasma canadense strain	21	Mycoplasma salivarium
4	Mycoplasma canadense strain ATCC	22	Mycoplasma salivarium strain MP
5	Mycoplasma canadense strain C	23	Mycoplasma sp.
6	Mycoplasma canis strain ATCC	24	Mycoplasma sp. Mycoplasmasp.
7	Mycoplasma canis strain Cb	25	Mycoplasma agalactiae
8	Mycoplasma canis strain Skotti A	26	Mycoplasma agalactiaePG
9	Mycoplasma cloacale	27	Mycoplasma agassizii strain Karina Fr. Tortoise
10	Mycoplasma corogypsi strain BV	28	Mycoplasma agassizii strain PS
11	Mycoplasma equirhinis strain ATCC	29	Mycoplasma alligatoris strain ATCC
12	Mycoplasma equirhinis strain M	30	Mycoplasma anatis
13	Mycoplasma gateae strain ATCC	31	Mycoplasma anatis strain KA
14	Mycoplasma gateae strain KDC	32	Mycoplasma anatis strain KC
15	Mycoplasma gateae strain V	33	Mycoplasma anseris
16	Mycoplasma hyopharyngis strain HB-F	34	Mycoplasma anseris alpingitidis strain MYCAV177
17	Mycoplasma hyopharyngis strain Nasal	35	Mycoplasma arginini
18	Mycoplasma leonicaptivi strain ATCC	36	Mycoplasma arginini clone V

No.	Mycoplasma Species/Strains	No.	Mycoplasma Species/Strains
37	Mycoplasma arginini strain ATCC	55	Mycoplasma californicum strain ATCC
38	Mycoplasma arthritisL	56	Mycoplasma californicum strain ST
39	Mycoplasma arthritis strain	57	Mycoplasma capricolum
40	Mycoplasma arthritis strain ATCC	58	Mycoplasma caviae strain ATCC
41	Mycoplasma bovis genitalium strain ATCC	59	Mycoplasma caviae strain G
42	Mycoplasma bovis genitalium strain MH	60	Mycoplasma citelli strain RGC
43	Mycoplasma bovis genitalium strain PG	61	Mycoplasma citelli strain RG-C
44	Mycoplasma bovirhinis	62	Mycoplasma citelli strain RGD-L
45	Mycoplasma bovis	63	Mycoplasma citelli strain RGD-S
46	Mycoplasma bovis HB	64	Mycoplasma collis strain ATCC
47	Mycoplasma bovis Hubei	65	Mycoplasma columbinasale strain ATCC
48	Mycoplasma bovis PG clone MU clone A	66	Mycoplasma columbinum
49	Mycoplasma bovis strain- (Del Giudice)	67	Mycoplasma columbinum strain FG
50	Mycoplasma bovis strain ATCC	68	Mycoplasma columborale strain
51	Mycoplasma bovis strain HEK-FDA	69	Mycoplasma columborale strain ATCC
52	Mycoplasma bovis strain Madison	70	Mycoplasma conjunctivae HRC/T
53	Mycoplasma bovis oculi strain ATCC	71	Mycoplasma conjunctivae strain Goa
54	Mycoplasma buteonis strain Bb/Tg	72	Mycoplasma conjunctivae strain Goat

No.	Mycoplasma Species/Strains	No.	Mycoplasma Species/Strains
73	Mycoplasma conjunctivae strain HRC	91	Mycoplasma flocculare strain Ms
74	Mycoplasma cottewii	92	Mycoplasma gallinaceum
75	Mycoplasma cricetuli	93	Mycoplasma gallinaceum strain
76	Mycoplasma crocodyli	94	Mycoplasma gallinaceum strain ATCC
77	Mycoplasma crocodyli MP	95	Mycoplasma gallinaceum strain F
78	Mycoplasma crocodyli strain ATCC	96	Mycoplasma gallinaceum strain R
79	Mycoplasma dispar strain ATCC	97	Mycoplasma gallinaceum strain SA
80	Mycoplasma edwardii strain	98	Mycoplasma gallinarum ATCC
81	Mycoplasma edwardii strain ATCC	99	Mycoplasma gallinarum strain ATCC
82	Mycoplasma elephantis strain ATCC	100	Mycoplasma gallinarum strain B (Dinter)
83	Mycoplasma equigenitalium strain ATCC	101	Mycoplasma gallisepticum strain F
84	Mycoplasma falconis ATCC	102	Mycoplasma gallisepticum strain JXA
85	Mycoplasma felifaucium strain PU	103	Mycoplasma gallisepticum strain JXB
86	Mycoplasma felis strain ATCC	104	Mycoplasma gallisepticum strain MG-HS
87	Mycoplasma fermentansJER	105	Mycoplasma gallisepticum strain ML
88	Mycoplasma fermentansM	106	Mycoplasma gallisepticum strain WSA
89	Mycoplasma fermentans strain ATCC	107	Mycoplasma gallisepticum strain WSB
90	Mycoplasma flocculare	108	Mycoplasma gallisepticum strain XA

No.	Mycoplasma Species/Strains	No.	Mycoplasma Species/Strains
109	Mycoplasma gallisepticum strain XZA	127	Mycoplasma hyorhinis isolate LuSIV
110	Mycoplasma gallisepticum strain XZC	128	Mycoplasma hyorhinis MCLD
111	Mycoplasma gallisepticum strain YMA	129	Mycoplasma hyorhinis strain ATCC
112	Mycoplasma gallisepticum strain YMC	130	Mycoplasma hyorhinis strain GDL
113	Mycoplasma gallopavonis strain ATCC	131	Mycoplasma hyosynoviae strain ATCC
114	Mycoplasma gallopavonis strain WR	132	Mycoplasma iguanae strain
115	Mycoplasma glycyphilum	133	Mycoplasma indienne
116	Mycoplasma gypis	134	Mycoplasma iners strain ATCC
117	Mycoplasma gypis strain ATCC	135	Mycoplasma iners strain D-E
118	Mycoplasma hominis ATCC	136	Mycoplasma iners strain PG
119	Mycoplasma hominis strain ATCC	137	Mycoplasma iners strain R-D
120	Mycoplasma hyopharyngis	138	Mycoplasma lagogenitalium strain ATCC
121	Mycoplasma hyopneumoniae	139	Mycoplasma leachii
122	Mycoplasma hyopneumoniae J	140	Mycoplasma leopharyngis
123	Mycoplasma hyopneumoniae strain ATCC	141	Mycoplasma lipofaciens strain ATCC
124	Mycoplasma hyorhinis ATCC:	142	Mycoplasma maculosum strain ATCC
125	Mycoplasma hyorhinis clone V	143	Mycoplasma maculosum strain Skotti B
126	Mycoplasma hyorhinis GDL	144	Mycoplasma meleagridis

No.	Mycoplasma Species/Strains	No.	Mycoplasma Species/Strains
145	Mycoplasma meleagridis strain ATCC	163	Mycoplasma phocicerebrale strain CSL S
146	Mycoplasma miroungirhinis	164	Mycoplasma phocirhinis strain ATCC
147	Mycoplasma moatsii strain MK	165	Mycoplasma phocirhinis strain CSL
148	Mycoplasma molare strain ATCC	166	Mycoplasma phocirhinis strain CSL-lung
149	Mycoplasma mycoides	167	Mycoplasma primatum strain ATCC
150	Mycoplasma neurolyticum strain ATCC	168	Mycoplasma primatum strain HRC
151	Mycoplasma neurolyticum strain CFA	169	Mycoplasma primatum strain I
152	Mycoplasma neurolyticum strain RN-F	170	Mycoplasma pullorum
153	Mycoplasma neurolyticum strain T	171	Mycoplasma pullorum strain ATCC
154	Mycoplasma opalescens	172	Mycoplasma pulmonis (strain UAB CTIP)
155	Mycoplasma orale	173	Mycoplasma pulmonis strain Ash (PG)
156	Mycoplasma orale strain MT	174	Mycoplasma putrefaciens
157	Mycoplasma ovipneumoniae strain ATCC	175	Mycoplasma salivarium
158	Mycoplasma oxoniensis	176	Mycoplasma simbae ATCC
159	Mycoplasma phocicerebrale ATCC	177	Mycoplasma sp. CSL-conjunctiva
160	Mycoplasma phocicerebrale strain	178	Mycoplasma sp. CSL-lung
161	Mycoplasma phocicerebrale strain ATCC	179	Mycoplasma sp. CSL-oral
162	Mycoplasma phocicerebrale strain Cheryl	180	Mycoplasma sp. Mirounga ES_AG_GEN

No.	Mycoplasma Species/Strains	No.	Mycoplasma Species/Strains
181	Mycoplasma sp. Mirounga ES-NAS	192	Mycoplasma synoviae
182	Mycoplasma sp. Mirounga ES-ORL	193	Mycoplasma synoviae ATCC
183	Mycoplasma sp. Phocoena C	194	Mycoplasma testudineum strain ATCC
184	Mycoplasma sp. Phocoena C--GEN	195	Mycoplasma testudineum strain BH
185	Mycoplasma spermatophilum	196	Mycoplasma verecundum
186	Mycoplasma spermatophilum strain (Jacobs)	197	Mycoplasma verecundum strain ATCC
187	Mycoplasma spermatophilum strain AH	198	Mycoplasma vulturii strain Gb-V
188	Mycoplasma spenis strain UCMJ	199	Mycoplasma yeatsii
189	Mycoplasma spumans	200	Mycoplasma zalophi strain CSL
190	Mycoplasma sturni ATCC	201	Mycoplasma zalophidermidis strain CS
191	Mycoplasma subdolum strain ATCC		

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