EZ-PCR™ Mycoplasma Detection Kit

Ready-to-use PCR kit for the detection of mycoplasma in cell cultures

Cat. No. 20-700-20 (20 reactions)

Store at: -20°C

Introduction

The EZ-PCR™ Mycoplasma Detection Kit is designed for routine screening and detection of mycoplasma - a common and dangerous contaminant of cell cultures and cell culture-derived biologics. This assay uses the precise method of polymerase chain reaction (PCR) to amplify a target sequence within the highly-conserved 16S rRNA coding region of the mycoplasma genome. A highly-specific and optimized primer set that allows for the detection of over 90 species of Mycoplasma, Acholeplasma, and Spiroplasma (see appendix for full list) is provided, creating a PCR assay with enhanced sensitivity and specificity designed with a detection limit of 10 CFU/mL.

The kit includes a ready-to-use PCR reaction mix (containing Taq DNA polymerase, dNTPs, and a highly-optimized primer set), PCR buffer solution, and a positive DNA template control. An internal amplification control (IC), a plasmid containing a non-mycoplasma-specific DNA sequence, and primer set is also included. The IC is simultaneously amplified and should be used with all PCR reactions, including the negative and positive controls. The IC rules out inhibition from biological material, among other assay malfunctions that can lead to false negatives.

Kit components

Component *(cap color)*	Description	Included volume
PCR reaction mix *(yellow)*	Optimized primer set, Taq polymerase, dNTPs	200 µL
Buffer solution *(blue)*	Allows for efficient Taq polymerase activity	1.0 mL
Positive template control *(red)*	Highly-conserved, mycoplasma-specific DNA sequence	20 µL
Internal control primer mix *(orange)*	Evaluates PCR integrity to rule out inhibition or malfunction	100 µL
Internal control DNA template *(purple)*		20 µL

Additional preparation needs

Equipment required
Thermocycler for PCR
Sterile microcentrifuge tubes
Agarose gel electrophoresis apparatus
Microcentrifuge
Positive displacement micropipette
Aerosol-preventive pipette tips

Reagents not included
2% agarose gel (with ethidium bromide)
100bp DNA ladder
Sterile water
Mineral oil (if required for thermocycler)

Storage

Store all components at -20°C
Note: Avoid repeated freeze/thaw cycles. When in use, always keep kit vials on ice.

Instructions for use

A. Test sample collection and preparation

To ensure best results, collect media test samples between 24 and 48 hours after the last medium exchange.

1. Remove buffer solution from storage, and thaw completely at room temperature. Store on ice during sample collection and preparation.
2. Transfer 1.0 mL cell culture supernatant into a 1.5 mL sterile microcentrifuge tube.
3. To remove cellular debris, centrifuge sample at 250 x g for 30 seconds.
4. Transfer the supernatant to a new 1.5 mL sterile microcentrifuge tube, leaving the pellet behind.
5. To sediment mycoplasma, centrifuge the supernatant at 15,000 to 20,000 x g for 10 minutes.
6. Carefully remove supernatant while keeping pellet intact (pellet may not be easily visible).
7. Resuspend the pellet with 50 µL of supplied buffer solution.
8. Mix thoroughly by vortexing, and briefly centrifuge to collect contents at the bottom of the tube.
9. Heat the resuspended sample at 95°C for 3 minutes.
10. The sample may be amplified by PCR immediately or stored at -20°C for later use.

B. PCR reaction set-up

Due to the high sensitivity and specificity of the EZ-PCR™ Mycoplasma Detection Kit, it is critical to avoid cross-contamination between samples. Sterile techniques should be used throughout the sample preparation process, including: changing pipette tips with each reagent, and keeping each reagent or sample covered. A clean and specifically-dedicated space for PCR is also desirable. To avoid false positive results, the use of sterile water, a positive displacement micropipette, aerosol-preventive filter tips, and gloves are also highly recommended. The positive DNA template control should be used last and should be kept separate at all times during preparation to avoid potential cross-contamination.

1. Thaw all components of the EZ-PCR™ Mycoplasma Detection Kit completely at room temperature. Store all components on ice once thawed.
2. Vortex kit reagents to mix, and briefly centrifuge each reagent to collect contents at the bottom of the tube.
3. Prepare the PCR reaction samples according to the preparation table below. Place each sample on ice once prepared.
4. Briefly vortex reaction samples to mix, and centrifuge to collect contents at the bottom of the tube.
5. *Optional*: If the thermocycler used requires mineral oil overlay to prevent evaporation during PCR denaturation temperatures, add 40 µL of mineral oil on top of the prepared PCR mix in each sample tube.
6. Maintain samples on ice and proceed to PCR amplification.

PCR reaction table

Component	Test sample(s)	Negative control	Positive control
Sterile water	29 µL	29 µL	33 µL
PCR reaction mix	10 µL	10 µL	10 µL
Internal control primer mix	5 µL	5 µL	5 µL
Internal control DNA template	1 µL	1 µL	1 µL
Buffer solution	--	5 µL	--
Test sample	5 µL	--	--
Positive template control	--	--	1 µL

Instructions for use, continued

C. PCR amplification

1. While samples are on ice, set thermocycler parameters as shown in the table below.
2. Place PCR samples in the thermocycler and initiate the programmed amplification settings.
3. After PCR amplification, samples are ready for gel electrophoresis.

Thermocycler parameters

	Temperature	Time	Cycles
Step 1	94°C	30 seconds	1 cycle
Step 2	94°C	30 seconds	35 cycles
	60°C	120 seconds	
	72°C	60 seconds	
Step 3	94°C	30 seconds	1 cycle
Step 4	60°C	120 seconds	1 cycle
Step 5	72°C	300 seconds (5 min)	1 cycle
Step 6	4°C	hold	--

D. Gel electrophoresis of amplified products

Note: A 2% agarose gel is critical for clear and accurate results.

1. Prepare a 2% agarose gel containing ethidium bromide (or other DNA stain). It is not necessary to add loading dye/buffer to the samples.
2. Carefully load 20 µL of 100bp DNA ladder, followed by each PCR sample in the gel lanes (see example gel lane set-up). The positive control should be handled last and run as the final sample in the gel.
3. Run the 2% agarose gel at 100V for 75 minutes (see FAQ section for additional techniques).
4. Analyze results to verify amplified product and bp size based on result interpretation section.

Example gel lane set-up:

Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7
100bp DNA Ladder	Sample 1	Sample 2	Sample 3	Negative Control	Positive Control	Optional: 100bp DNA Ladder

E. Result interpretation

The internal control band at 357bp should be present in all samples. If the internal control band is absent, PCR inhibition may have occurred, and the test is invalid - refer to the FAQ section below. A test sample that is positive for the presence of mycoplasma shows a distinct band at 270bp. The negative control lane should only show the internal control band at 357bp.

Sample	Band size	Band significance
Test Sample	270bp 357bp	Mycoplasma positive Internal control positive
Test Sample	357bp	Mycoplasma negative Internal control positive
Test Sample	270bp	Mycoplasma positive Internal control negative (see troubleshooting guide)
Any Reaction	<100bp	Primer dimerization may have occurred (see troubleshooting section)
Any Reaction	No band at 357bp	PCR inhibition may have occurred (test may be invalid, see FAQ section)

Result example #1

Note: *Images courtesy of WiCell Research Institute.*

Sample	Band size	Band significance
Sample 4	270bp	Mycoplasma positive
	357bp	Internal control positive
Samples 1, 2, 3, 5, & 6	357bp	Mycoplasma negative Internal control positive
Negative Control	357bp	Mycoplasma negative Internal control positive
Positive Control	270bp	Mycoplasma positive
	357bp	Internal control positive

Result example #2

Sample	Band size	Band significance
Sample 1	270bp	Mycoplasma positive
	357bp (faint)	Internal control positive (faint due to PCR competition)
Sample 5	270bp (faint)	Mycoplasma positive (faint)
	357bp	Internal control positive
Samples 2, 3, 4, & 6	357bp	Mycoplasma negative Internal control positive
Negative Control	357bp	Mycoplasma negative Internal control positive
Positive Control	270bp	Mycoplasma positive
	357bp	Internal control positive

Troubleshooting

Issue	Potential cause	Next steps
The internal control band is faint or missing, but my test sample is positive (band at 270bp)	PCR competition may have occurred	High amounts of mycoplasma within the sample can lead to PCR competition, resulting in a faint internal control band at 270bp, or no internal control band at all. Try testing a more dilute concentration of the sample.
The negative control also shows a positive band (at 357bp)	The kit reagents may have become contaminated	Use the same kit to run another test with a positive control and negative control only. If the negative control still shows a positive band, check sterility practices and dispose of kit.
Bands appear lower than 100bp	Primer dimerization may have occurred	While this often does not affect the sensitivity of the test, it could be due to old reagents that have degraded over time. A new kit may be needed to ensure clear and easily interpreted results.
Bands appear “smeared”	The collected sample may contain too many cellular bi-products	Confirm that the test sample was not taken longer than 48 hours after media exchange. Try testing a sample within 24 hours of media exchange.

Additional FAQs

Does the cell culture need to be confluent before collecting samples?
No. Due to the high sensitivity of the EZ-PCR™ Mycoplasma Detection Kit, it is not necessary to culture the cells to confluency for an accurate readout.

What happens if samples are collected for mycoplasma testing more than 48 hours after the last media exchange?
If cell culture media is older than 48 hours when collected, cellular byproducts can accumulate that can inhibit the PCR reaction. In this case, it is best to replace media and collect for PCR between 24 and 48 hours later. If a sample collected after the 48-hour timeframe must be used, DNA extraction is recommended prior to PCR amplification.

How can I be sure that a faint positive band means my sample is mycoplasma positive?
To know for sure, a sample producing a faint positive band may be re-tested. If a faint band persists in the subsequent assay, the sample should be considered mycoplasma positive. If the faint band no longer appears in the subsequent assay, the sample is negative.

How definitive are the given gel electrophoresis parameters?
Running the 2% agarose gel at 100 volts for 75 minutes is a recommendation but may vary depending on the apparatus. If recommendations for specific gel electrophoresis setups are needed, one option is to prepare and run a pre-made 2% E Gel agarose gel (Thermo Fisher) according to protocol with the appropriate program on the E-Gel® iBase™ Power System (Thermo Fisher) to run for 26 minutes.

Appendix

Specificity of the EZ-PCR™ Mycoplasma Detection Kit

The primers were aligned with the National Center for Biotechnology (NCBI) database and inspected for homologies within the mycoplasma 16S rRNA target region. This primer sequence is unique to mycoplasma; consequently, human, mammalian, bacterial, or other potentially contaminating DNA will not produce a positive result. The following are specific species with relevant sequence homologies that will be detected by the EZ-PCR™ Mycoplasma Detection Kit:

Mycoplasma fermentans	*Spiroplasma citri*	*Mycoplasma glycophilum*
Mycoplasma hyorhinis	*Acholeplasma pleciae*	*Mycoplasma gypis*
Mycoplasma arginini	*Acholeplasma palmae*	*Mycoplasma hyopharyngis*
Mycoplasma orale	*Acholeplasma granularum*	*Mycoplasma iguanae*
Mycoplasma salivarium	*Mycoplasma adleri*	*Mycoplasma indiense*
Mycoplasma hominis	*Mycoplasma agalactiae*	*Mycoplasma iners*
Mycoplasma pulmonis	*Mycoplasma agassizii*	*Mycoplasma lagogenitalium*
Mycoplasma arthritidis	*Mycoplasma alvi*	*Mycoplasma leonicaptivi*
Mycoplasma bovis	*Mycoplasma alkalescens*	*Mycoplasma leopharyngis*
Mycoplasma pneumoniae	*Mycoplasma anseris*	*Mycoplasma lipophilum*
Mycoplasma pirum	*Mycoplasma auris*	*Mycoplasma maculosum*
Mycoplasma capricolum	*Mycoplasma bovirhinis*	*Mycoplasma microti*
Acholeplasma laidlawii	*Mycoplasma buccale*	*Mycoplasma moatsii*
Mycoplasma synoviae	*Mycoplasma buteonis*	*Mycoplasma mobile*
Mycoplasma hyosynoviae	*Mycoplasma californicum*	*Mycoplasma molare*
Mycoplasma genitalium	*Mycoplasma canadense*	*Mycoplasma mustelae*
Mycoplasma bovigenitalium	*Mycoplasma canimucosale*	*Mycoplasma oxoniensis*
Mycoplasma gallinarum	*Mycoplasma caviae*	*Mycoplasma phocicerebrale*
Mycoplasma meleagridis	*Mycoplasma citelli*	*Mycoplasma phocidae*
Mycoplasma iowae	*Mycoplasma collis*	*Mycoplasma phocirhinis*
Mycoplasma falconis	*Mycoplasma columbinasale*	*Mycoplasma ravipulmonis*
Mycoplasma penetrans	*Mycoplasma columbinum*	*Mycoplasma simbae*
Mycoplasma cloacale	*Mycoplasma columborale*	*Mycoplasma sphenisci*
Mycoplasma spermatophilum	*Mycoplasma conjuctivae*	*Mycoplasma spumans*
Mycoplasma opalescens	*Mycoplasma cricetuli*	*Mycoplasma sturni*
Mycoplasmaprimatum	*Mycoplasma elephantis*	*Mycoplasma sualvi*
Mycoplasma faucium	*Mycoplasma equigenitalium*	*Mycoplasma subdolum*
Mycoplasma caprine	*Mycoplasma cynos*	*Mycoplasma testudineum*
Mycoplasma agalactica	*Mycoplasma edwardii*	*Mycoplasma turnidae*
Mycoplasma timone	*Mycoplasma equirhinis*	*Mycoplasma verecundum*
	Mycoplasma gallopavonis	*Mycoplasma zalophi*
	Mycoplasma gateae	*Mycoplasma zalophidermidis*

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