Ver. HB221123

GMyc-PCR Mycoplama Test Kit

Product description

Cell culture is a common experiment in life science research. Unlike other commonly used experimental methods, cell culture is a dynamic continuous process, and cells often respond to manipulation errors or contaminants that often exhibit abnormal cell states or medium appearance. If it is contaminated by mycoplasma, the cell morphology has no obvious change, and it is easy to be overlooked. It is often not found until the pollution is very serious. There may be hundreds of mycoplasmas on the contaminated cell membrane, these mycoplasmas compete for nutrients and release toxic metabolites, seriously affecting the experimental results.

Studies have shown that at least 20 kinds of mycoplasma can contaminate cells, among which the most common are: oral Mycoplasma (*M. orale*), Mycoplasma arginine (*M. arginini*), Mycoplasma hyorhinis (*M. hyorhinis*), Mycoplasma fermentum (*M. fermentans*), Mycoplasma hominis (*M. hominis*), Mycoplasma salivarius (*M. salivarium*), Mycoplasma pulmonary (*M. pulmonis*) and Mycoplasma pear (*M. pirum*). The mycoplasma contamination rate of cultured cells ranges from 4% to 92%. The sources of contamination include the working environment, the operator itself (some mycoplasmas are normal flora of the human body), culture medium, serum, cell cross-contamination, experimental equipment and used Contamination of the original tissue or organ from which cells were prepared.

Identifying the underlying cause of problems during cell culture is a difficult and time-consuming task, where any sudden changes should be suspected, and good testing practices and regular testing for mycoplasma contamination are necessary. There are many methods for the detection of mycoplasma, such as direct culture, DNA fluorescence staining, ELISA and PCR methods.

GMyc-PCR Mycoplasma Detection Kit mainly uses PCR method to detect Mycoplasma infection of various biological materials (such as cell culture, experimental animal secretions, animal serum, etc.). It combines several advantages: sensitive, specific, rapid and can be detected directly with cell culture supernatants. This product detects mycoplasma in biological materials such as cultured cells by PCR method. The primers used are designed according to the conserved region of the 16S-23S rRNA sequence of mycoplasma, and only specifically amplify the mycoplasma DNA, with high detection sensitivity and specificity. PCR amplification and electrophoresis analysis only takes a few hours, and the operation is convenient and simple.

Components

Components No.	Name	40601ES10 (10 assays)	40601ES20 (20 assays)
40601-A	GMyc-1st PCR Mix	250 μL	2×250 μL
40601-B	GMyc-2nd PCR Mix	250 μL	2×250 μL
40601-C	Positive Control Template^{N}	20 μL	20 μL

【Notes】1. When not in use for a long time, it can be stored frozen at -80°C.

2. The PCR reaction is extremely sensitive. In order to prevent false positives, a positive control is added at the end

when adding samples.

Shipping and Storage

Shipping in ice packs. Store at -15°C ~ -25°C, the shelf life is 18 months. If it is not used for a long time, please keep it away from light.

Instructions

This PCR reaction is nested PCR. After the first round of PCR is completed, the reaction product is taken as the second round of PCR template. The results were analyzed according to the presence or absence of electrophoresis bands of the two rounds of PCR products and the size of the fragments (the first round of positive control PCR band size was 448 bp, and the second round of positive control PCR band size was 304bp). After 3-6 days of cell culture, the supernatant was directly taken for PCR reaction. To exclude cell culture media from inhibiting the PCR reaction, an equal amount of cell supernatant should be added to the positive control.

1. First Round PCR

1.1 Fully melt the GMyc-1st PCR Mix, and prepare the reaction solution according to the table below:

Reagents	Test Group	Positive Control	Negative Control
GMyc-1st PCR Mix	25 μL	25 μL	25 μL
Sample	4 μL	4 μL	
ddH_2O	21 μL	21 μL	25 μL
Positive Control Template [N]		1 μL	

【Notes】 In order to prevent the pollution of the Positive Control Template, please add the Positive Control Template to the positive control group after adding the experimental group and the negative control group.

1.2 Perform the PCR reaction under the following conditions:

94 °C	5 min	
94 °C	30 s	30~35 cycles
58 °C	30 s	
72 °C	30 s	
72 °C	7 min	

Second Round PCR

1. Fully melt the PCR Mix, and prepare the reaction solution according to the table below:

Reagents	Test Group	Positive Control	Negative Control
GMyc-1st PCR Mix	25 μL	25 μL	25 μL
ddH_2O	24 μL	24 μL	24 μL
1st PCR reaction (1000-fold dilution) [N]	1 μL	1 μL	1 μL

【Notes】 The first-round PCR reaction solution needs to be diluted 1000 times before it can be used for the second-round PCR template.

2. Perform the PCR reaction under the following conditions:

94 °C	5 min	
94 °C	30 s	30~35 cycles
58 °C	30 s	
72 °C	30 s	
72 °C	7 min	

After the reaction, take 7 μL of PCR reaction solution (without adding loading buffer) and directly perform 1.5% agarose gel electrophoresis to confirm the PCR amplification product.

Recommended Frequency of Use	
New cells enter the lab	Must check
Before storage in liquid nitrogen	Must check
Regular routine	Check once a month
After discovering contamination	Check once a week
Find abnormal cells	Check at any time

Species	First Round PCR	Second Round PCR
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

Notes

1. Please read this manual carefully before using this reagent.
2. Talk as little as possible during operation, because the oral cavity also contains mycoplasma, which may cause sample contamination and cause false positives; during the entire detection process, the preparation of the reaction system, sample processing and sample addition, and PCR amplification should be carried out in different areas. to avoid cross contamination.
3. During the experiment, the reagents in the kit components should be fully thawed and mixed well before use (violent shaking is prohibited during mixing, it is only necessary to invert up and down several times for mixing);
4. After adding all the reagents to the reaction tubes, it should be amplified on the PCR machine as soon as possible to avoid the formation of too many dimers.
5. Antibiotics such as penicillin and streptomycin in cell culture will not affect the detection results of this product. If

the user needs to further improve the detection sensitivity, it is recommended that the cells be cultured in the absence of antibiotics such as penicillin and streptavidin for 2-3 days and then sampled for detection.

6. For your safety and health, please wear a lab coat and disposable gloves for operation.

7. For research use only!