Mycoplasma Detector

Catalog: CM1001

Introduction:

Mycoplasma contamination is a common issue in mammalian cell culture, potentially leading to inaccurate experimental results. Since 2013, high-impact journals like "Nature" have required mycoplasma testing for articles involving cell culture. This product utilizes a unique isothermal gene amplification technology, designed to provide a rapid, simple, and highly sensitive method for mycoplasma detection.

Product Advantages:

1. **Rapid Detection:** The entire detection process takes only 1 hour, significantly reducing the weeks required by traditional culture methods.

2. **Easy Operation:** No PCR inhibitors have been found in cell culture fluids to inhibit isothermal gene amplification, generally eliminating the need for sample pre-treatment.

3. **High Sensitivity:** The sensitivity of isothermal gene amplification surpasses that of conventional PCR methods with 30 cycles.

4. **Intuitive Results:** No electrophoresis needed; results can be directly observed by color change of the indicator.

5. **Low Equipment Requirements:** The entire detection process requires only a water bath, eliminating the need for a PCR machine, electrophoresis tank, or gel imaging system.

Product Limitations:

1. **dentification Rate:** The mycoplasma identification rate is relatively low, identifying approximately 99% of mycoplasma species, with about 1% risk of false negatives.

2. **Interference Factors:** High concentrations of serum, blood products, divalent ions, and EDTA may interfere with the colorimetric results but can be removed by centrifugation and washing.

Detection Range:

This kit can detect at least the following 13 types of mycoplasma:

Mycoplasma hyorhinis	Mycoplasma fermentans
Mycoplasma arginini	Mycoplasma hominis
Mycoplasma orale	Mycoplasma salivarium
Mycoplasma pirum	Acholeplasma laidlawii
Mycoplasma agalactiae	Mycoplasma bovis
Mycoplasma buccale	Mycoplasma arthritidis
Mycoplasma pulmonis	

The first 8 types are the most common mycoplasma contaminants in cell culture, accounting for approximately 98% of contaminated cells, while the above 13 types account for about 99% of contaminated cells.

Mycoplasma Detector provides a rapid, simple, and highly sensitive method for mycoplasma detection, suitable for laboratories needing quick detection of mycoplasma contamination in cell culture. Despite certain limitations, its high efficiency and simplicity make it an indispensable tool in cell culture laboratories.

Components

(1) Solution 1: 1150 µL (for 50 tests)

(2) Solution 2: 55 µL

(3) Solution 3: Indicator, 38 µL

(4) Positive Mycoplasma DNA: 50 µL

Materials required but not provided

(1) 0.2 mL clear flat-cap PCR tubes

(2) 200 µL and 10 µL filter tips

(3) Mineral oil

(4) PCR instrucment or water bath

Detection Procedure

Sample Preparation:

Method 1: Direct Detection(This method is less sensitive than Method 2 and cannot remove potential interferents, which may cause colorimetric interference)

1. Take 150 µL of the sample into a 1.5 mL centrifuge tube.

2. Centrifuge at 1000 rpm (approximately 150 g) for 5 minutes in a standard benchtop centrifuge.

3. Take 100 µL of the supernatant for mycoplasma detection, discard the remaining 50 µL containing cell pellets.

4. The sample with cells removed by centrifugation can be directly detected or detected after heat treatment (samples are more stable after heat treatment).

5. Heat Treatment: Heat at 95°C for 5 minutes (can be done in a PCR machine), briefly centrifuge (1000 g, 5 seconds), and take the supernatant for detection.

Method 2: Simple Centrifugation and Washing(Recommended method. Can concentrate mycoplasma to improve detection sensitivity, remove most interferents, and reduce colorimetric interference)

1. Depending on the required concentration factor, take 100-1500 µL of the sample into a 1.5 mL centrifuge tube.

2. Centrifuge at 1000 rpm (approximately 150 g) for 5 minutes in a standard benchtop centrifuge.

3. Transfer the supernatant to another centrifuge tube, discard the cell pellet.

4. Continue to centrifuge the supernatant at 13000 rpm (approximately 16000 g) for 5 minutes.

5. Carefully remove the supernatant, retaining 3-5 µL of liquid at the bottom. Resuspend the pellet in 20-50 µL of 5 mM Tris-HCl (pH 8.0-8.8) or deionized water (Tris-HCl is recommended for long-term storage; deionized water is suitable for short-term detection).

6. The resuspended sample can be directly detected or detected after heat treatment (samples are more stable after heat treatment).

7. Heat Treatment: Heat at 95°C for 5 minutes, briefly centrifuge (1000 g, 5 seconds), and take the supernatant for detection.

Note:

1. The cell culture supernatant referred to here is not the centrifuged supernatant after cell digestion with trypsin, but the supernatant of the culture medium from adherent cells cultured for at least 2 days (no trypsin digestion is required, and the centrifuged supernatant after trypsin digestion cannot be used for detection) or suspension cell culture medium.

2. The purpose of low-speed centrifugation in this step is to remove mammalian cells to eliminate unnecessary interference. Therefore, the centrifugal force must be strictly controlled at 150-200 g. Under this centrifugal force, mammalian cells will precipitate while mycoplasma will not. Incorrect use of higher centrifugal force may cause mycoplasma to precipitate as well, leading to false-negative results.

3. If the collected cell culture supernatant samples are not tested immediately, please store them in a -20°C or -80°C freezer, not at room temperature or in a 4°C refrigerator. Samples can be stored at -20°C for at least one month and long-term at -80°C. Additionally, to save detection costs, samples collected at different times can be stored in a -20°C or -80°C freezer and tested together later.

Preparation of the Reaction System【It is strongly recommended to use filter tips for all operations in this step to avoid contamination of the reagents. Before proceeding, please carefully read the precautions in the following text】:

(1) Take Solution 1 and Solution 3 out of the -20°C freezer and thaw them at room temperature. Before opening the cap, flick the centrifuge tube of Solution 1 to bring the solution on the tube wall to the bottom, and centrifuge Solution 3 (approximately 3000 rpm, 1 minute), then pipette up and down to mix well. [Note: Solution 1 and Solution 3 must be pipetted up and down to mix well after each thawing before use, and centrifuge Solution 2 and Solution 3 before opening the cap]. Prepare the reaction system according to Table 1:

	Volume per Sample (μL)	Total Number of Samples	Total Volume (μL)
solution 1	21.5	N	21.5 × N × 1.06
solution 2	1	N	1 × N × 1.06
solution 3	0.5	N	0.5 × N × 1.06

Table 1: Preparation of the Isothermal Reaction System

Example:

1. If there are 8 samples to be tested (plus 1 negative and 1 positive control), the total number of samples is 10. The total volume of Solution 1 is 21.5 × 10 × 1.06 = 227.90 μL. The total volume of Solution 2 is 1 × 10 × 1.06 = 10.60 μL. The total volume of Solution 3 is 0.5 × 10 × 1.06 = 5.3 μL. Mix Solution 1, Solution 2, and Solution 3 thoroughly.

2. If you intend to use up one kit at a time, you can prepare it as follows: directly add 50 μL of Solution 2 and 25 μL of Solution 3 into the entire tube of Solution 1 (1075 μL), and mix well. If used up in one go, one kit can test approximately 48-49 samples.

Note:

1. An additional 6% of the total volume is prepared to prevent pipetting errors and ensure that the reaction liquid in each reaction tube is sufficient (customers can adjust this proportion if they find it inappropriate).

2. After using Solutions 1 and 3, please return them to the -20°C freezer for storage.

3. Solution 2 must always be stored in the -20°C freezer (Solution 2 will not freeze at -20°C) and cannot be stored at room temperature.

4. Centrifuge Solutions 2 and 3 before opening the cap.

(2) After mixing the prepared isothermal reaction system thoroughly by pipetting, aliquot 23 µL into each 0.2 mL PCR tube.

Note:

1. Ensure that the reaction liquid volume in each tube is consistent. If the volume of the reaction liquid in the last tube is insufficient, it can be used as a negative control tube.

2. PCR tubes should be of good transparency.

(3) For the negative control tube (Negative), you can add 2 µL of sterile water (prepared by yourself, or it can be omitted); add 2 µL of the sample to be tested into the test tube (Test); add 2 µL of positive Mycoplasma DNA into the positive control tube (Positive). The total volume of the reaction liquid is 25 µL.

Note:

1. Do not centrifuge the screw-cap tube containing positive Mycoplasma DNA at high speed. Before opening the cap, simply hold and flick it with your fingers. Before pipetting, mix thoroughly by pipetting up and down. If it is accidentally centrifuged at high speed, ensure to mix thoroughly by pipetting up and down before pipetting, otherwise, the positive control result may be abnormal.

2. It is best to separate the room where the reaction system is prepared from the room where sample pre-processing, adding positive control DNA, and sample DNA are performed.

Reaction (Choose one of the following methods for the reaction):

(1) Water bath reaction (Note: This product can only be used with a regular water bath containing water; it cannot be used in a dry metal bath, as the temperature of a dry metal bath is very inaccurate.): Add 25 µL of mineral oil to each reaction tube to prevent evaporation, cover the tubes, insert the reaction tubes into a floating rack with holes, and place it into a regular water bath preheated to 61°C for an accurate 60-minute reaction.

Note: Before the reaction, the water bath must be preheated to 61°C before placing the reaction tubes. Additionally, use a mercury thermometer to measure the water temperature to ensure the accuracy of the water bath temperature. The enzyme used in this kit is very sensitive to temperature, and the temperature difference between the instrument display and the actual temperature must not exceed 1°C.

(2) PCR machine reaction: For laboratories with a PCR machine, it is strongly recommended to perform the reaction on a PCR machine to accurately control the reaction time and temperature. The PCR machine parameters are as follows: 61°C for 60 minutes; 10°C, forever; hot lid temperature, 105°C.

Note: When performing the reaction on a PCR machine with a hot lid, there is no need to add mineral oil to the reaction tubes (most modern PCR machines are equipped with a hot lid).

Result Interpretation: After the 61°C reaction for 60 minutes, immediately remove the reaction tubes and place them at room temperature. Using a white paper or a white foam box (preferably white foam) as the background, the detection result can be determined by the color change of the reaction solution. If the solution is blue-green, it indicates mycoplasma contamination; if it is purple-red, it indicates no mycoplasma contamination (as shown in Figure 1).

Note:

1. The reaction time at 61°C must be accurately timed; exceeding the specified reaction time may lead to false positives (do not exceed by more than 5 minutes). If the kit has expired or other unexpected circumstances affect enzyme activity, and it is found that after 60 minutes of reaction at 61°C, the color difference between positive and negative is not obvious, consider continuing the reaction for an additional 10 minutes at 61°C in a water bath or PCR machine. However, if the color difference between positive and negative is clear after 60 minutes of reaction at 61°C, do not continue the reaction, as this may result in false positives. The above is a remedial measure for unexpected situations.

2. If, after the reaction, the color of certain samples falls between positive and negative (normally this should be very rare; if it occurs frequently, the samples may contain substances that interfere with the normal indication effect and must be removed first. The removal method is described in the precautions section later), these samples can continue to react at 61°C for an additional 10 minutes. After 10 minutes, if the color still differs from the positive control, the sample should be judged as negative. It is recommended to retest such suspicious samples using the same kit or other kits with different principles (such as the company's "PCR Mycoplasma Detection Kit," "Luminescence Mycoplasma Detection Kit," and "Probe Mycoplasma Detection Kit").

3. After the reaction, do not open the caps of the reaction tubes, as this may cause contamination of the testing environment. After interpreting the results, seal them in a zip-lock bag and dispose of them in a trash bin in a different room.

4. Different batches of products, as well as factors such as photography, may cause the colors of the positive and negative controls after the reaction to slightly differ from those shown in Figure 1 of this manual (for example, the negative control color may appear light red or purple-red). As long as there is a significant color difference between the positive and negative controls after the reaction, the reaction is considered successful.

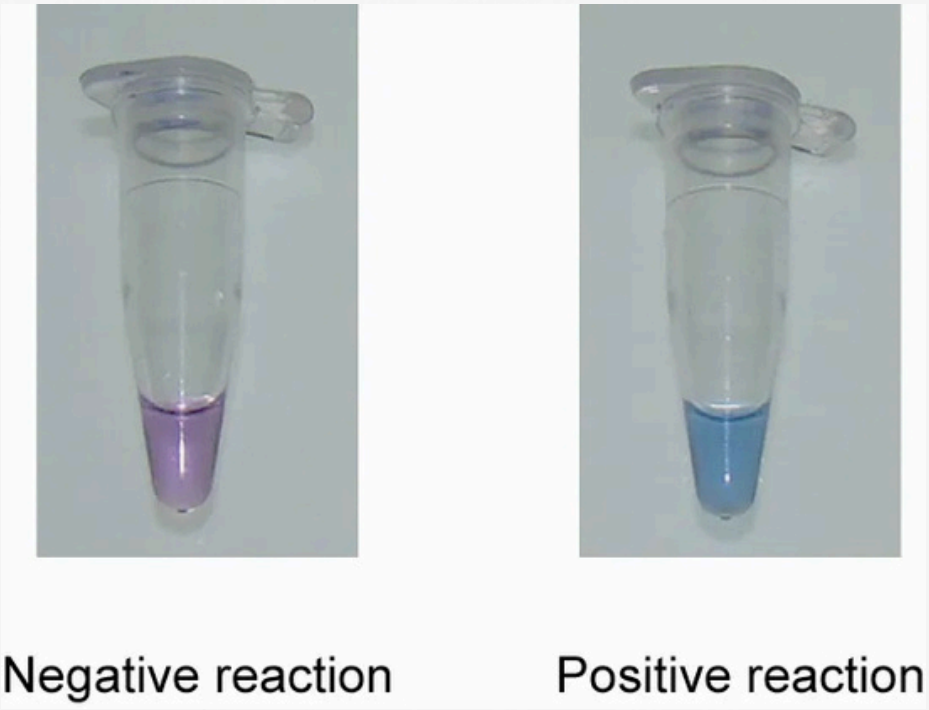

Figure 1

Precautions

1. Before the reaction, ensure that the colors of the negative control, positive control, and all test samples are basically consistent after adding the samples. If any sample shows a color that is significantly different from the negative and positive controls upon addition, it indicates the presence of substances that can interfere with the normal indication effect of this system, and these substances must be removed. This phenomenon has occurred when testing CHO serum-free culture media and some blood products (such as albumin solution, plasma, serum, etc.). Common culture media such as DMEM, MEM, F12, 1640, etc. (which can contain 10% serum) generally do not exhibit this phenomenon. Removal methods:

(1) Centrifugal washing: Take 1 mL of cell supernatant or test sample (e.g., albumin solution, plasma, serum), centrifuge at 13000 rpm for 5 minutes, and remove 950 µL of the supernatant; add 950 µL of PBS, centrifuge again, and remove 950 µL of the supernatant; add 950 µL of PBS for the third time, carefully remove all the supernatant (to avoid disturbing the bottom sediment, retain 3-5 µL of liquid at the bottom), resuspend the sediment in 20-50 µL of 5 mM Tris-HCl, pH 8.0-8.8 (samples can be stored long-term, recommended) or deionized water (samples are less stable and only suitable for testing on the same day or shortly thereafter), and mix thoroughly by pipetting. The resuspended samples can be tested directly or after heat treatment (samples are more stable after heat treatment). The heat treatment process is as follows: heat at 95°C for 5 minutes (can be transferred to a PCR tube and heated in a PCR machine), then perform a brief centrifugation (1000 g, 5 seconds), and use the supernatant for testing. Note that centrifugal washing may result in the partial loss of mycoplasma, and if the mycoplasma content in the sample is very low, it may lead to false negatives.

(2) Use "Mycoplasma Genomic DNA Extraction Kit" to extract mycoplasma DNA for testing. DNA extraction can remove all possible interfering substances and concentrate mycoplasma DNA by 10-100 times.

2. Since the various enzymes used in isothermal amplification strongly depend on divalent ions in the solution, chelating agents such as EDTA in the test samples should only be present in trace amounts. If you plan to extract mycoplasma DNA using a kit (recommended to use "Mycoplasma Genomic DNA Extraction Kit"), elute and dissolve the DNA with deionized water or a 5 mM Tris-HCl buffer (pH 8.0-8.8) without EDTA.

3. Precautions to prevent DNA contamination:

(1) The room for preparing the isothermal reaction system (for this kit, operate in a regular room with windows and good ventilation. Do not perform this step in a closed cell culture room, as the probability of mycoplasma contamination is high) should be separate from the rooms used for sample preprocessing, adding positive control DNA, and sample DNA.

(2) It is strongly recommended to use filter tips for pipetting related solutions and positive mycoplasma samples. If filter tips are not available, at least use newly opened tips.

(3) Ensure that the pipettes used are free from residual mycoplasma. It is best to use newly purchased pipettes. If new pipettes are not available, use pipettes that have not been previously used for cell culture, as pipettes used for cell culture are likely to be contaminated with mycoplasma-containing culture medium (e.g., accidentally aspirating mycoplasma-contaminated culture medium into the pipette body during cell culture). Mycoplasma adsorbed in the pipette can cause unnecessary false positives.

(4) Carefully handle all types of tips, centrifuge tubes used for sample preprocessing, and tips used for aspirating positive control DNA and test sample DNA. Place them in a sealed bottle containing half a bottle of water. After all samples have been aspirated, cover the bottle to prevent the volatilization of positive DNA, which could contaminate the environment and cause false positives.

(5) During the entire operation process, it is best not to speak, as the human mouth and saliva contain mycoplasma.

(6) After the reaction, do not open the reaction tube cap, as this may contaminate the testing environment. After judging the results, seal the tubes in a ziplock bag and dispose of them in a trash can in another room.