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Mycoplasma Detection Kit – DigitalTest v2.0

Description

When a cell culture system is contaminated by mycoplasma, the mycoplasma's specific metabolic enzymes will degrade medium components, such as amino acids, and produce metabolites which are then secreted into the cell culture medium.

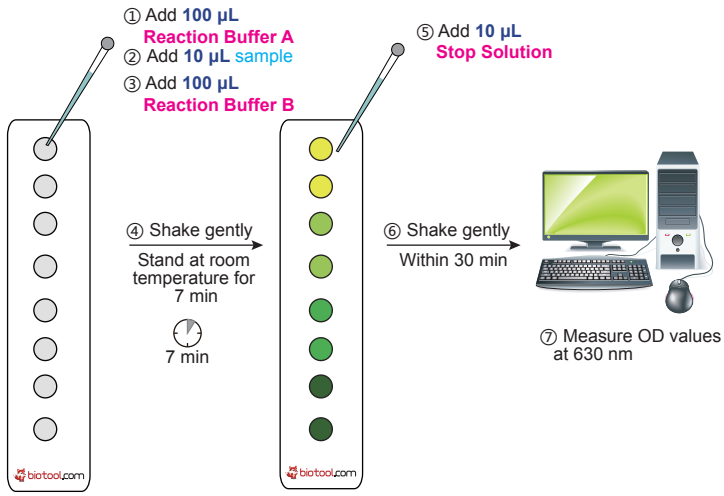
If the sample to be examined contains these metabolites, the reaction system (including the sample, the reaction strip, and the reaction solution) will turn greenish-blue in color. The concentration of metabolites produced by mycoplasma is proportional to the darkness of the color, which directly indicates the amount of mycoplasma in the sample. Measure OD values of the sample and negative control, calculate the difference, and compare the OD difference with the threshold constant 0.105 (see Protocol below) to identify the presence of any mycoplasma contamination.

Components

Component	B39132 (8 test × 10)	B39135 (8 test × 50)	B39138 (8 test × 500)
Test Strip	10	50	500
Reaction Buffer A	8 mL	8 mL x 5	8 mL x 50
Reaction Buffer B	8 mL	8 mL x 5	8 mL x 50
Stop Solution	0.8 mL	0.8 mL x 5	0.8 mL x 50
Positive Control *	0.2 mL	0.2 mL x 5	0.2 mL x 50

* Positive Control is the metabolites of mycoplasma, not mycoplasma organism.

Protocol



! All materials and samples should be at room temperature: 22-28°C.

1). Open the test strip.

2). Add 100 µL Reaction Buffer A to each well. All the buffers and samples should be added by multichannel pipettes.

3). The 1st well should be left blank with no testing sample, while 10 µL negative control should be added to the 2nd well. The negative control must be unused cell culture medium and should undergo the same incubation procedure as the cell culture supernatant to be tested. Do NOT use water as negative control. Other wells could be used for additional samples (cell culture supernatant) or positive control. Shake gently after adding.

4). Add 100 µL Reaction Buffer B to all wells. Shake gently.

5). Let stand at room temperature for 7 min.

6). Add 10 µL Stop Solution to each well. Shake gently.

7). At Em 630 nm, set the OD value of the blank well as zero, and then measure OD values. Calculate the value of OD_{sample} (ODs) minus $OD_{negative\ control}$ (ODc).
   a) If ODs – ODc ≥ 0.105, the sample is contaminated by mycoplasma;
   b) If ODs – ODc < 0.105, the sample is free of mycoplasma.

Note

1. Because this method is based on detecting metabolic products of mycoplasma, we recommend performing the test after 48 hr continuous cell culturing.

2. The optimal reaction condition is at room temperature: 22-28°C. The reaction temperature must be over 18°C. We recommend warming the kit to room temperature before starting the test, especially in cold environments.

3. A blank well is required. It reflects the exact value of reaction solution excluding the culture media.

4. The color changing reaction stops when adding Stop Solution. The colors are stable for 30 min, and should be recorded within this time range.

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5. The following samples can NOT be examined by this method:
   a) Any kind of serum-free media;
   b) F12 cell culture medium;
   c) DMEM/F12 cell culture medium.
   These media contain high concentration of interfering components. These components can inherently cause color changing, leading to false results.

6. Product studies show that the color differences between negative, weak positive, and positive samples are most obvious within the first 7 min after adding Reaction Buffer B. There is a linear relationship between color depth and the analyte concentration during this period.

7. The material used to coat the test plate may change color when exposed to air. Only open the test plate immediately before use.

8. For research use only. Cannot be used for clinical purposes.

Limitations of This Method

1. This kit cannot distinguish the species of mycoplasma, but can effectively detect the presence of many types of mycoplasma.

2. If the cell culture system is weakly contaminated by mycoplasma (less than 10 mycoplasma copies / uL cell culture supernatant), the result may show weakly positive. We suggest re-testing the mycoplasma contamination after appropriate extension of cell incubation time (24-48 h).

Storage

• Store at 4-25°C for 12 months.

Trouble Shooting

Q1: How can I test if fresh cell culture medium is contaminated by mycoplasma?
Prepare two dishes, one contains fresh cell culture medium only, another one contains cell culture medium and mycoplasma removal reagent (such as Biotool Mycoplasma Removal Kit, Cat. No. B39182). Incubate both at 37°C for 48 hours, and then perform test according to the protocol above. If OD value of the dish with medium only is bigger than the dish with medium and removal reagent, it suggests that this medium contains viable mycoplasma. Attention, this method is invalid when species of mycoplasma are resistant to removal reagent.

Q2: For the same sample, why are the results of PCR methods POSITIVE, while the results of this detection kit are NEGATIVE?
The results of PCR methods may come out as positive even if the mycoplasma is dead. The results of this detection kit may be negative, because of the following reasons: PCR methods detect the existence of mycoplasma via amplification of the 16S rRNA sequence. Regardless of whether the mycoplasma is alive or not, if there is presence of mycoplasma 16S rRNA, the result will be positive. This kit detects mycoplasma based on the metabolites produced by mycoplasma, so this kit will only detect the presence of viable mycoplasma.

Q3: For the same sample, why are the results of PCR methods NEGATIVE, while the results of this detection kit are POSITIVE?
PCR method detects the existence of mycoplasma via amplification of 16S rRNA sequences. The mycoplasma species that can be detected by PCR methods closely depend on the primer sequence. This rationale explains how the PCR methods can only detect a limited variety of mycoplasma. This kit detects mycoplasma contamination based on metabolites produced by many types of mycoplasma. When the primers used in the PCR method cannot amplify the target region, the result of PCR method is negative, even if there is viable mycoplasma in the sample.

Q4: Can this mycoplasma detection kit be used to detect contaminations of cell culture supernatants stored at 4°C which were collected previously?
Yes, but samples must be warmed to room temperature before testing. The metabolites produced by the mycoplasma are stable when stored at 4°C. This kit can be used to reliably detect metabolites found in cell supernatant stored at 4°C for up to 5 days post-collection.

Q5: Why is it not recommended using H2O as negative control?
The cell culture medium contains serum. During the serum preparation process, metabolites of mycoplasma may still be retained, even if the mycoplasma has been removed. Therefore, when using this mycoplasma detection kit, it's highly recommended to use the same cell culture medium as the negative control, and the same batch of cell culture medium is the best negative control.