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Myco-Qtech Kit

(Extraction)

Quantity: 50 Reactions
Storage: 2-8 °C
Shipment: Ice gel
Cat. No.: LG0092

For Research Use Only. Not for use in diagnostic procedures.

Myco-Qtech Kit (Extraction)

Introduction

Myco-Qtech Kit (Extraction) efficiently extracts Mycoplasma genomic DNA from most types of contaminating Mycoplasma. When combined with our Myco-Qtech kit (Detection), this extraction protocol gives labs the best chance to accurately identify suspect cultures.

Kit Component

	Volume	Storage
Myco Extraction Buffer	2×50 ml	2-8 °C
Nuclease-free Water	3 ml	2-8 °C

Shipping and Storage Condition

The Myco-Qtech Kit (Extraction) is shipped on ice gel and should be stored at 2-8 °C immediately upon receipt.

Equipment Required (not included)

1. 1.5 ml sterile (DNase and RNase free) microcentrifuge tubes

2. Pipettes with corresponding filter tips (10, 100, 1000 µl)

Sample Preparation

1. The cells must have been grown without antibiotics for at least 3 subcultures.

2. The cells should be kept in culture for at least 48-72 hours prior to Mycoplasma detection.

3. Collect the sample once the cells have reached at least 80% confluence.

DNA Extraction

1(a). Suspension cells: collect up to 3×10^6 cells in a centrifuge tube. Pellet the cells by centrifugation for 5 min at 300 ×g. Discard the supernatant. Wash the cells once with PBS, centrifuge for 5 min at 300 ×g, and remove the supernatant. Proceed to step 3(a).

1(b). Adherent cells: Remove the growth medium from a culture plate. Rinse cells with PBS to remove residual medium. Discard PBS. Detach the cells from the culture plate by scraping in an appropriate volume of PBS or by trypsinization. Transfer 3×10^6 cells into a centrifuge tube and pellet them by centrifugation for 5 min at 300 ×g. Wash the cells once again with PBS, centrifuge for 5 min at 300 ×g, and remove the supernatant. proceed to step 3(a).

1(c). Cell culture supernatant: Transfer 1 ml of cell culture supernatant into a centrifuge tube. proceed to step 3(b).

2 (Optional). Add 10 µl of Myco-Q Positive Control that is included in Myco-Qtech Kit (Detection) into a portion of collected test sample in step 1 as spiked sample.

3(a). Resuspend the cell collected in step 1(a) or 1(b) in 1 ml Myco Extraction Buffer by pipetting to obtain a uniform suspension. Then Centrifuge the tube at 1000 ×g for 10 min at 4 °C.

3(b). Centrifuge the tube containing cell culture supernatant at 1000 ×g for 10 min at 4 °C.

4. Transfer the supernatant into a clean tube and then centrifuge at 13000 ×g for 15 min at 4 °C. A small pellet may be formed at the bottom of the tube in this step.

5. Carefully remove and discard the supernatant. Add 1 ml Myco Extraction Buffer to the pellet, mix well by pipetting, and incubate at 2-8 °C in a refrigerator for 10 min. Then centrifuge at 13000 ×g for 15 min at 4 °C.

6. Carefully remove and discard the supernatant. Then incubate the tube for 30-45 min at room temperature in inverted position to dry the pellet.

7. Dissolve the pellet in 30 µl of pre-warmed (56 °C) Nuclease-free Water.

8. Use the purified DNA immediately in Real-time PCR using Myco-Qtech Kit (Detection) or store at -20 °C.

Be your Best

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