DNA PrepMate - M

Description

This is a one tube protocol to minimize carry-over contamination and sample losses.

Tuberculosis is caused by *Mycobacterium tuberculosis, Mycobacterium africanum,* and *Mycobacterium bovis. M. tuberculosis* is widely distributed in nature and the illness it causes is a problem of major medical importance. Isolation of the pathogenic bacteria and their subsequent identification by biochemical testing usually requires 4-8 weeks.

In addition, the radiometric BACTEC system, the immunodiagnostic approach, and the radioisotope method using labeled DNA probes have several drawbacks; e.g., low sensitivity and time-consuming, technically difficult protocols.

Recently, the PCR technique, which can amplify specific target DNA sequences, has been applied in the rapid amplification and identification of many microorganisms, including *M. tuberculosis.*

The insertion sequence IS6110 (normally found in the bacterial chromosome, which probably facilitates higher sensitivity in PCR) was selected as a target sequence for PCR-based detection.

DNA PrepMate-M kit is extraction kit without organic solvent for *Mycobacterium tuberculosis*.

PROTOCOL

I - Extraction of total DNA from samples :

I - 1 Sputum

I - 2 CSF / Urine

I - 3 Pleural fluid

I - 4 Tissue

I - 1 Sputum

1. Solubilize 500ul of sputum taken from suspected patient with 50ul of hydrolysis buffer.
2. Vortex for 5 min & centrifuge at 6,000 rpm for 5 min.
3. Discard the supernatant and suspend the pellet with 500ul of 1x Washing Solution.
4. Centrifuge tube at 6,000 rpm for 2 min. And repeat the 3 and 4 steps twice.
5. Discard the supernatant completely using the yellow tip and resuspend the pellet with 50ul of Lysis Buffer.
6. Add mineral oil enough for overlay and heat each tube in Microwave for 5 min. (or boil for 20 min at least) *
7. Centrifuge at 13,000 rpm for 10 min.

5 - 10ul of supernatant solution is suitable for 20ul PCR reaction.

Note *: If the overlay is insufficient or omitted, the old solution will evaporate and DNA & protein will coagulate.

I - 2 CSF / Urine

1. Centrifuge tube containing 20 - 50mL of Urine or 2 - 5mL of CSF at 13,000 rpm for 10 min.
2. Discard the supernatant carefully and suspend the pellet with 500ul of 1x Washing Solution.
3. Centrifuge at 6,000 rpm for 2 min. Repeat steps 2 and 3 twice.
4. Discard the supernatant completely and resuspend the pellet with 50ul of Lysis Buffer.
5. Add mineral oil enough for overlay and heat each tube in microwave for 5 min. (or boil for at least 20 min)
6. Centrifuge at 13,000 rpm for 10 min. Use 5 - 10ul of supernatant for PCR.

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I - 3 Pleural fluid

1. Solubilize 2 - 5mL of Pleural fluid with 100ul of hydrolysis buffer.
2. Centrifuge at 13,000 rpm for 10 min.
3. Discard the supernatant and suspend the pellet with 500ul of 1x Washing Solution
4. Centrifuge at 6,000 rpm for 2 min. Repeat 3 and 4 step twice.
5. Discard the supernatant completely and resuspend the pellet with 50ul of Lysis Buffer.
6. Add mineral oil enough for overlay and heat each tube in microwave for 5 min. (or boil for at least 20 min)
7. Centrifuge at 13,000 rpm for 10 min. Use 5 - 10ul of supernatant for PCR.

I - 4 Tissue

1. Add 300ul of SE Buffer (75mM NaCl, 25mM EDTA, pH 8.0), 0.5 % SDS and 50ug/ml Proteinase K to the sample and incubate for 12 - 24 hours at 37°C (or for 2 hours at 50°C)
2. Add 400ul of phenol / chloroform (1 : 1) and vortex very well.
3. Centrifuge at 13,000 rpm for 15 min at 4°C.
4. Transfer the supernatant to the fresh tube and add an equal volume chloroform / isoamyl alcohol (24 : 1) and mix very well.
5. Centrifuge at 13,000 rpm for 15 min at 4°C.
6. Add 0.1 volume of 3M NaOAc (pH 5.2) and 2 volume ice-cold Ethanol, incubate for 1- 2 hours at -20°C.
7. Centrifuge at 13,000 rpm for 15 min at 4°C.
8. Discard the supernatant and wash with 1mL 70 % ice-cold ethanol.
9. Centrifuge at 13,000 rpm for 3 min at 4°C.
10. Repeat 8 and 9 step once.
11. Discard the supernatant and dry the pellet with vacuum concentrator, resuspend the pellet with 50ul of TE Buffer (10mM Tris-Hcl, pH 8.0, 1mM EDTA, pH 8.0) containing 20ug/mL RNase and incubate for 1 hour at 37°C.
12. Use 2 - 5ul of DNA for PCR. DNA obtained by this procedure is stable for 60 days at 4°C or more than 1 year at - 20°C .

Usage of 8-MOP

8-Mop Solution was included in DNA PrepMate-M for PCR reaction, not for DNA extraction. It helps to remove the false positive results caused by contaminated templates by crosslink formation between DNA strands on UV light. Add 8-Mop solution to *AccuPower*™ PCR PreMix at final PCR step. After PCR reaction, the tube should be exposed on 254nm of UV illuminator for 5min.

Ordering Information

Cat. No.	Description
K-3020	DNA PrepMate-M includes Hydrolysis buffer 30 ml 10X Washing buffer 100 ml Lysis buffer 15 ml 8-MOP solution 10 ml