Bacterial DNA extraction from raw milk using Ribospin™ Pathogen/TNA kit

Experimental Conditions

Materials Required

- Ribospin™ Pathogen/TNA (50 preps: 341-150 / 250 preps: 341-152)
- Absolute ethanol (C_2H_6O, CAS No.: 64-17-5, ≥99.0%)
- 1.5 ml or 2.0 ml microcentrifuge tube
- Vortex mixer
- Centrifuge (Max. speed 14,000 rpm or ≥10,000 x g)
- Pipette & sterile pipette tips
- Suitable protector (e.g., lab coat, disposable gloves, goggles, etc.)

Sample Information

- Sample type: raw milk
  (with the spiked vaccine of *Mycoplasma* spp.)

- Sampling: after collecting fresh raw milk samples, immediately inject the vaccines of *Mycoplasma* spp. (10^0 to 10^{-4} dilution) in the samples and extract the bacterial DNA.

- Extraction conditions
  - Sample amount: 1 ml
  - Elution volume: 50 µl

Protocol

Before Starting

- Before using for the first time, add absolute ethanol (ACS grade or better) into Buffer RB1, Buffer RBW, and Buffer RNW as indicated on the bottle.
- If a precipitate has formed in Buffer KL, heat to dissolve at 56 °C before use.

Sample Preparation

1. Transfer 1.0 ml of the raw milk to a 1.5 or 2.0 ml microcentrifuge tube.
2. Centrifuge at 10,000 x g above for 5 min at room temperature and discard the supernatant containing fat and liquid layer.
3. The next step is according to **Ribospin™ Pathogen/TNA protocol**.

Ribospin™ Pathogen/TNA Protocol (Raw Milk)

*** For more details and methods, please refer to the handbook of Ribospin™ Pathogen/TNA.**

Lysis	1. Add 200 µl Buffer SL and resuspend the pellets. 2. Add 20 µl of Proteinase K solution and 200 µl of Buffer KL. Vortex vigorously and incubate at 20-25 ˚C for 5 min.
Binding	3. Add 300 µl Buffer RB1, pulse-vortex to mix, and spin down briefly. 4. Transfer the mixture to Column Type P and centrifuge at 10,000 x g above for 1 min.
Washing	5. Add 600 µl Buffer RBW to Column Type P and centrifuge at 10,000 x g above for 1 min. 6. Add 600 µl Buffer RNW to Column Type P and centrifuge at 10,000 x g above for 1 min. 7. Centrifuge at full speed for 1 min to remove the residual wash buffer. 8. Transfer Column Type P to a new 1.5 ml microcentrifuge tube.
Elution	9. Add 50 µl of Nuclease-free Water to Column Type P and incubate at room temperature for 1 min. 10. Centrifuge at full speed for 1 min.

Table 1. Brief protocol of GeneAll® Ribospin™ Pathogen/TNA kit for bacterial DNA purification from the raw milk.

Result

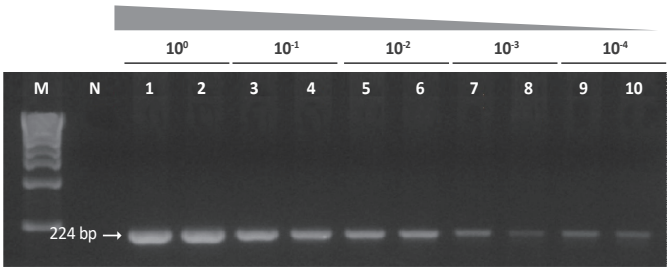

Figure 1. Conventional PCR amplification result of *Mycoplasma* spp. gene using DNA extracted from raw milk as template.
M: GENESTA™ 1 kb DNA ladder with 5X loading dye (GA-100)
N: negative control (nuclease-free water)
Lanes 1, 2: amplified bacterial DNA
Lanes 3, 4: amplified bacterial DNA (10^{-1} dilution)
Lanes 5, 6: amplified bacterial DNA (10^{-2} dilution)
Lanes 7, 8: amplified bacterial DNA (10^{-3} dilution)
Lanes 9, 10: amplified bacterial DNA (10^{-4} dilution)

※ Electrophoresis conditions: 2% agarose gel, 150 V, 15 min, 2 µl loading