**MagNA Pure DNA Isolation from Sputum**

**Materials/Equipment**

Sputolysin (CALBIOCHEM 560000)

UltraPure Water (Invitrogen 10977-015)

0.2 µm syringe filter (Thermo Scientific 723-2520)

3 mL syringe (BD 309585)

Pipet tips 1000 µL (Genemate P-1237-1250)

Pipet tips100 µL (Neptune BT100)

1.6 mL Eppendorf tubes (Neptune 3745.X)

0.6 mL Eppendorf tubes (Neptune 3735.A. X)

MagNA Pure Bacterial Lysis Buffer (BLB) (Roche 04659180001)

Proteinase K (Qiagen 19133)

Glass bead tubes, 0.1 mm (MOBIO 13118-50)

MagNA Pure Nucleic Isolation Kit (Roche 03730964001)

Lysostaphin (Sigma L7386-5MG or -15MG)

Lysozyme (Sigma L6876-1G)

Bead Beater (BioSpec)

Water bath at 37°C

Heat blocks at 65°C, 95°C

Eppendorf 5415D tabletop centrifuge

Vortex Genie 2 mixer

Tissue homogenizer (Omni International TH-01)

MagNA Pure Compact Instrument (Roche 03731146001)

**Lysozyme Working Solution**

1. Weigh out 100 mg Lysozyme powder.

2. Add 1 mL UltraPure water and vortex well to dissolve.

3. Store at 4°C. The Lysozyme Working Solution is stable for 1 month at 4°C.

**Lysostaphin Working Solution**

1. Dissolve 15 mg Lysostaphin in 1.5mL UltraPure water.

2. Dispense into aliquots and store at -20°C. Avoid multiple freeze-thaw cycles. The Lysostaphin Working Solution is stable for 6 months at –20°C.

**Sputum Prep Protocol**

1. Thaw sputum samples on ice.
2. Dilute sputolysin 1:10 in water. Filter sterilize with 0.2 µm syringe filter. Keep stock solution in dark.
3. Aliquot 175 µL diluted sputolysin to 1.5 mL Eppendorf tubes. Label with sputum numbers.
4. Add 175 µL thawed sputum to labeled tubes, rinsing pipet tips with sputolysin to ensure all the sputum is eluted from tip.
5. Vortex and incubate sputum+sputolysin at 37°C for up to 30 min, or until sample is fairly uniform.
6. Remove samples from heat bath and vortex. Add 315 µL BLB to sputum+sputolysin and invert several times to mix.
7. Add 176 µL of Lysozyme Working Solution to 88 µL Lystostaphin working solution to make Lysozyme+Lysostaphin (LZ/LS) master mix.
8. Add 30 µL LZ/LS master mix to samples. Invert several times to mix.
9. Incubate at 37°C for 30 min.
10. If sample does not become uniform, transfer to a 5 mL tube and homogenize with tissue homogenizer.
11. Transfer 650 µL sputum+sputolysin+BLB+LZ/LS to bead tube.
12. Beadbeat 1 min on MAX setting.
13. Spin 1 min at 15,000 x g.
14. Add 35 µL Proteinase K and invert to mix.
15. Incubate 10 min at 65°C. (This is a good time to check that the MagNA Pure machines are turned on)
16. Beadbeat 1 min on MAX.
17. Spin 1 min 15x000 x g.
18. Incubate 10 min at 95°C.
19. Transfer 400 µL to MagNA Pure sample tube, avoiding beads in the bottom of the tube. Use the bead tube caps while transferring samples to the MagNA Pure instrument.
20. Proceed to MagNA Pure Compact Protocol.

**MagNa Pure Compact Protocol**

1. Main Menu – Click “Run”
2. Insert Reagent Cartridges.
3. Select “DNA\_Bacteria” protocol, elution volume 100 µL (no internal control).
4. Insert tip trays into the tip rack. Visually confirm presence of tips with filters in each position. Hold tip trays upright to prevent tips from falling out. Proceed to Screen 3.
5. Enter the Sample Names for each sample in the order they will be run. Remove caps and insert sample tubes into row 1 of the tube rack (labeled “S”). Confirm tubes all sit flush in the rack. Proceed to Screen 5 (skips screen 4 because there are no internal controls.)
6. Scan the barcode for each elution tube and insert into the elution tube rack (labeled “E”.)
7. Close the front cover, confirm drop catcher present, confirm data, and start the run.
8. After the run has ended, confirm all channels “PASS”.
9. Cap and remove elution tubes. Transfer to -20°C.