GSure® Sputum DNA Mini Kit

#G45273 50 preparations

Store at Room Temperature

Procedure:

1. Take 30-50 ul of sputam sample (freshly collected) in a fresh microfuge tube. When collecting sample with cotton swab: Take 300 µl GDSP1 buffer in a microfuge tube, dip the cotton swab in buffer, roll vigorously while pressing the swab in the tube wall.
2. Add 250 µl Buffer GDSP1 in sputum sample when working with crude sputam sample. Incubate the tube at 70^0C for 15 minutes and vortex after every 2 minutes.
3. Add 250 µl Buffer GDSP2 and mix by inverting the tube 4–6 times. Place the tube at 70^0C again for another 15 minutes.
4. Add 350 µl Buffer GDSP3 and invert the tube immediately. Shake vigorously to mix the solutions, **DO NOT VORTEX AT THIS STAGE**. Vortex may cause shearing of genomic DNA.
5. Centrifuge for 10 minutes at 13,000 rpm (~8500xg) in a table-top microcentrifuge. A compact pellet will form.
6. Apply the supernatant to the Gmini Spin Column by decanting or pipetting. ***Avoid mixing of cell debris with the supernatant*** *as* this may clog Gmini spin Column thus lowering the DNA yield.
7. Centrifuge at 13,000 rpm (~8500xg) for 30–60 s. Discard the flow-through.
8. Wash Gmini Spin Column by adding 600µl Buffer Membrane Wash Buffer and centrifuging for 30–60 s as previously.
9. Discard the flow-through.
10. Repeat washing step.
11. Discard the flow-through, and centrifuge for an additional 2 minutes to remove residual wash buffer from membrane.

> ***This step is extremely important to ensure complete removal of ethanol. Presence of ethanol in purified DNA may inhibit subsequent enzymatic reactions.***

12. Place the Gmini Spin Column in a clean 1.5 ml microcentrifuge tube (not provided). To elute DNA, add 50 µl Nuclease-free Water to the center of each Gmini Spin Column, let stand for 1 minute, and centrifuge for 1 minute at maximum speed (~8500Xg) on a table top microcentrifuge.
13. Discard the column and collect the eluted DNA present in microcentrifuge tube.

- If required, increase the volume of GDSP1, GDSP2 and GDSP3 accordingly.
- **If any sediment found in the isolation buffers, warm the containers until it dissolves.**

Kit Content:

GDSP1 Buffer	: 15ml
GDSP2 Buffer	: 15ml
GDSP3 Buffer	: 20ml
Membrane Wash Buffer	: 30ml
Spin Column	: 50pcs.
Nuclease-free Water	: 3ml

Reconstitution of Membrane Wash Buffer:

Before using the kit for first time, add 45ml of absolute ethanol (molecular biology grade) with the provided wash buffer. Mix thoroughly by shaking. Once ethanol added, tighten the cap properly after each use.