DNA DIAGNOSTIC

PROTOCOL –4N6FLOQSwabs

This protocol describes the use of COPAN 4N6FLOQSwab with Active Drying System (Cat. 4500C), for sample to be tested with DNA Diagnostic PCR kits.

Sample collection:

1. *SURFACES*: Remove the swab from the tube and moderately wet the tip with **Swab Buffer** (do not saturate the swab tip). Swab across the surface multiple times, turning the swab tip regularly.
   LIQUID SAMPLE: Treat the sample with bronopol (0.2 to 0.4 mg/mL). Remove the swab from tube and dip the tip in the sample, but do not oversaturate the tip (avoid dripping of sample in tube).
2. Insert the swab back into the tube. Make sure that the lid is properly closed. Mark/write on the tube for sample identification.
3. Store and send the swab in tube at ambient temperature.
   Note: The sample will dry out on the swab tip, due to a desiccant in the swab lid. The sample is stable for at least 4 weeks, when tested with DNA Diagnostic kits.

Sample elution and DNA extraction:

4. Add **750uL Swab Elution Buffer** in a **2mL Deep Well plate** (or 2mL tube).
5. Remove the swab from the tube. If using deep well plates, also remove the swab tip on the plastic stick from the swab lid. Insert the swab tip into the deep well (or 2mL tube) containing Swab Elution Buffer.
6. Incubate 10 – 120 minutes at 37 °C.
7. Stir deep well plate with swabs on 96-well vortexer at 500 rpm for 2 minutes. Make sure that the liquid does not spill out of the wells.
   If using single 2mL tubes, stir the swab around in the liquid and press the swab against the well wall to press out liquid.
8. Remove and discard the swab.

From this step, the protocol is the same as the DNA Diagnostic Mastit 4 'DNA purification' protocol (step 3 and on).

9. Spin Deep Well Plate (or 2mL tubes) at 5000xg for 5 min. Remove supernatants from the top with tips connected to a vacuum system. Be careful not to touch or remove the bacterial pellets.
10. Add 1 mL Wash Buffer to each well/tube. Cover with sealing tape/close tube.
11. Spin at 5000xg for 5 min. Remove supernatants from the top with tips connected to a vacuum system. Be careful not to touch or remove the bacterial pellets.
    It is important to remove the supernatant completely, or leave a maximum of 10 µL supernatant.
12. Prepare fresh Lysis-I Mix by adding 6 µL *Mix I additive* to 54 µL *Lysis buffer-I* and mix.

Lysis-I Mix	1 reaction	9 reaction	100 reactions
Lysis Buffer-I	54 µL	486 µL	5400 µL
Mix I additive	6 µL	54 µL	600 µL
TOTAL	60 µL	540 µL	6000 µL

13. Add 60 µL Lysis-I Mix, to each pellet and cover with sealing tape (or close tube). Vortex the Deep Well plate/tube at least 10 seconds, or continue until pellet is homogenized (for some samples, pipetting up-and-down might be necessary). Spin quickly to bring Lysis mix to the tube bottoms (e.g. 10 seconds at 1000xg).
14. Remove caps from 96 well plate with clear 0.2 mL tubes.
15. Use an 8-channel pipette with filter tips and transfer of all 60 µL from each well of the Deep Well Plate into the corresponding tubes in the 96 well plate (clear 0.2 mL). Close tubes with the caps. Avoid cross contamination. Use one new tip per tube.
16. Incubate the 96 well plate at 37°C for 20 min.
17. Incubate the 96 well plate at 95°C for 15 min.
18. Cool the 96 well plate on ice for 5 min

    Note: The incubation at 37°C and 95°C can be done using a PCR instrument programmed: 37°C for 20 min. → 95°C for 15 min. → 4°C for 5 min.
19. Centrifuge the 96 well plate/tube at 5000xg for 5 min. at room temperature. Note it is important to use a 96 tube support for the 96 well plate during centrifugation.
20. Carefully remove the caps from the 96 well plate/tubes. Use an 8-channel pipette with filter tips to transfer 5 µL of each aqueous phase directly to the corresponding tubes of the 96 well plate with qPCR Master Mix in step 24.
21. The remaining purified DNA can be stored at -20°C for long time storage. After storage, thaw and vortex samples, and continue from step 19 (centrifuge).

qPCR analysis

22. Take a 96 Well Plate or 8-strip with qPCR Master Mix (DNA Diagnostic qPCR master mixes only – e.g. Mastit4, TBC4, Salmonella Velox, ect.) from -20°C, place on ice for five minutes and spin 20 seconds at 1000xg to bring qPCR Master Mix to the tube bottoms.
23. Discard the seal from the 96 Well Plate with qPCR Master Mix and place the 96 well plate on ice.
24. Transfer 5 µL purified DNA from step 20 to each of the corresponding tubes in the 96 well plate containing qPCR Master Mix.
25. Carefully close the qPCR tubes with a new optical lid. Spin the tubes briefly before transfer of the 96 Well Plate qPCR reactions to the qPCR instrument. Note it is important to keep the optical lids clean.
    Note: It is important to keep Master Mix on ice while loading template.

Please refer to the protocol of the selected DNA Diagnostic qPCR kit for further instructions.