REF RFSS-S1-05

Processing Instructions of BuccalFix RNA swab samples prior to RNA Isolation

Equipment & Reagents:

Supplied with kits:

- RFX Precipitation Reagent (store at room temperature).
- Proteinase K, 20 mg/ml (reconstitute lyophilised powder supplied with an appropriate volume of RNAse-free water prior to use, using the table below). Store reconstituted liquid at 4°C.

Proteinase K	Volume of H_2O to add
2.2 mg	110 µL
11 mg	550 µL
22 mg	1100 µL

To be supplied by user:

- RNAse-Free Water.
- Microcentrifuge tubes (1.5ml/2.0ml).
- Water/Dry Baths, preheated to 60°C and 90°C.
- Vortexer.
- Microcentrifuge (RCF ≥ 12,000 x g).

Processing Steps:

1. Gently vortex the swab collection tube to mix. Remove a 300-500µl aliquot of sample into a clean, RNase-free 1.5ml or 2ml tube.

2. Add 1/25 volume (12-20µl) of Proteinase K solution and vortex gently to mix.

3. Incubate aliquot at 60°C for 60 minutes, then heat @ 90°C for 15 minutes and cool to room temperature. Incubation time at 60°C can be reduced to 30 mins if required.

4. Add 1/25 volume (12-20µl) of RFX Precipitation Reagent. Vortex gently and incubate on ice for 10 minutes.

5. Spin at 12,000 x g for 3 minutes to pellet precipitate.

6. Carefully remove the supernatant into a clean, RNase-free 1.5ml or 2ml tube, taking care not to disturb the pellet. Discard the pellet.

7. Immediately add one volume of RNA lysis buffer from your RNA isolation kit of choice to the supernatant. Mix gently by inversion, then proceed with RNA isolation as per your isolation method protocol.