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Protocol status: Working We use this protocol and it's working

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Elution of nasal lining fluid collected via nasosorption

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ABSTRACT

This procedure outlines the materials and processes for eluting nasal lining fluid collected using a Mucosal Diagnostics FX-i nasosorption device. This protocol has been successfully used to elute nasal lining fluid for both DNA extraction for 16s rRNA sequencing & metagenomics, and for proteomics using the SomaScan assay.

GUIDELINES

This protocol should be performed in a sterile environment to reduce cross contamination between nasosorption devices, to enable metagnomic DNA extraction and analyses

MATERIALS

- Phosphate Buffered Saline (PBS) (#10010023)
- Tween 20 (Sigma, #P9416-50ML)
- Corning SpinX columns (Sigma, #CLS9301)
- 2mL Corning microfuge tubes (Sigma, #CLS3213)
- Sterile forceps (two pairs per swab)
- 1.6mL sterile microfuge tubes

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Making elution buffer

- 1 Add [M] 0.05 % volume of Tween20 to PBS in a sterile environment to reduce cross-contamination
- 2 Store at 4 °C until required

Elution of nasal lining fluid

30m 30s

- 3 Thaw nasosorption device in cryotube on ice
- 4 Add \perp 300 μ L of elution buffer to a labelled 2mL microcentrifuge tube and place on ice
- Remove the synthetic absorptive matrix (SAM) from the nasosorption device using sterile forceps by tearing it from the handle and place in the microcentrifuge tube containing elution buffer
- 6 Vortex the microcentrifuge tube for 00:00:30

30s

7 Using a new pair of sterile forceps, remove the SAM from the elution buffer, and place into a spinX column on a new 2mL microcentrifuge tube

- 8 Add the elution buffer from the first tube onto the SAM in the spinX column
- 9 Centrifuge the SAM and spinX column at 16000 x g for 00:30:00 at 4 °C
- 10 Discard the spinX column and SAM
- Aliquot the eluate into labelled microtubes, and measure total protein concentration using the assay of your choice (Pierce BCA assay, Qubit protein assay etc)

30m