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Macherey-Nagel Nucleospin 96 Food protocol for bee pollen

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ABSTRACT

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1 UV sterilize supplies for 2 96 well plates worth of extractions: 4 50mL centrifuge tubes, 2 15mL centrifuge tubes, zirconia beads, 2 96 deep well plates and clear strip caps, 2 s-blocks, 2 96 well elution plates, 14 1000uL tip boxes, 2 200uL tip boxes, 2 10uL tip boxes, 6 reagent troughs, and 6 96 well microplates

2 Lysis Buffer CF **Macherey Nagel Catalog #740946** Aliquot into sterile centrifuge tube and warm in **65 °C** water bath. You will need to aliquot **30 mL** per 1/2 plate (48 samples)

3 Proteinase K **Macherey Nagel Catalog #740506** Add **540 µL** to each warmed **30 mL** Lysis Buffer CF **Macherey Nagel Catalog #740946** aliquot and invert gently to mix

4 Lysis Buffer CF **Macherey Nagel Catalog #740946** Add **560 µL** + Proteinase K **Macherey Nagel Catalog #740506** solution to each sample

Each sample will receive **550 µL** Lysis Buffer CF **Macherey Nagel Catalog #740946** and **10 µL** Proteinase K **Macherey Nagel Catalog #740506**

5 Place in tissue lyser and run at **10 Hz** for **00:01:00** 1m

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Make sure pollen ball is removed from bee leg after lysing. If still attached, repeat this step, increasing Hz if needed

TissueLyser II
Bead Mill
QIAGEN **85300**

6 Centrifuge **3220 x g, 00:01:00** 1m

Eppendorf™ 5810R Centrifuge
Centrifuge
Eppendorf **02-262-8187**

7 Using sterile tweezers, remove bee leg, rinse with 200 proof ethanol, and place leg into labeled sterile microcentrifuge tube. Sterilize tweezers between each use with flame. Leave leg tubes open in fume hood until remaining ethanol has evaporated, then store at **-80 °C**

If extracting pollen not attached to bee leg, skip steps 5-7

8 Add ~ **100 µL** zirconia beads to each pollen sample

9 Place in tissue lyser and run at **24 Hz** for **00:01:30** , rotate plates 180 degrees, and lyse again at **24 Hz** for **00:01:30** 3m

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TissueLyser II
Bead Mill
QIAGEN 85300

10 Place in **65 °C** water bath for **00:30:00** 30m

Incubation time may be increased up to overnight if extraction of DNA from pollen during lysis was not sufficient

11 Centrifuge **3220 x g, 00:35:00** 35m

12 Transfer **300 µL** of supernatant into 96 deep well plate

Samples may be stored at **-20 °C** after this step

13 Add **300 µL** (or equal volume)
Binding Buffer C4 **Macherey Nagel Catalog #740366.250**
and **300 µL** (or equal volume) 200 proof ethanol

Binding Buffer C4 **Macherey Nagel Catalog #740366.250** and ethanol may be combined ahead of time. Check note on reagent bottle. If already combined, add **600 µL** Binding Buffer C4 **Macherey Nagel Catalog #740366.250** /EtOH

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Binding Buffer C4 **Macherey Nagel Catalog #740366.250** solution. with EtOH added may be stored at room temperature for up to 1 month

Binding Buffer C4 **Macherey Nagel Catalog #740366.250** contains guanidine salt - do not mix with bleach

14 Vortex samples until thoroughly combined

15 Centrifuge **1500 x g** for **00:00:30** 30s

Do not centrifuge at a higher g-force or for a longer duration - this will precipitate out DNA

16 Place food binding plate onto s-block and transfer sample to food binding plate. Seal with gas-permeable foil

17 Centrifuge **3220 x g, 00:09:00** , discard flowthrough 9m

18 While centrifuging, aliquot out **12 mL** per plate of Elution Buffer CE **Macherey Nagel** and place in **70 °C** water bath

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19 Wash Buffer CQW **Macherey Nagel Catalog #740313.125**

Add **500 µL** and seal

Wash Buffer CQW **Macherey Nagel Catalog #740313.125** contains guanidine salt - do not mix with bleach

20 Centrifuge **3220 x g, 00:04:00** , discard flowthrough 4m

21 Wash Buffer C5 **Macherey Nagel Catalog #740931**

Add **900 µL** , do not seal

22 Centrifuge **3220 x g, 00:20:00** , discard flowthrough 20m

23 Incubate binding plate **37 °C** for **00:30:00** 30m

24 Place binding plate over elution block. Add **100 µL** pre-heated Elution Buffer CE **Macherey Nagel** directly onto center of each binding plate filter membrane

25 Incubate room temperature **00:05:00** 5m

26 Centrifuge **3220 x g, 00:04:00** 4m

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27 Aliquot entirety of product into 3 96 well microplates per DNA plate

28 Store at **-20 °C** (short term) to **-80 °C** (long term)

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