

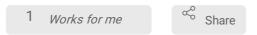


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Macherey-Nagel Nucleospin 96 Food protocol for bee pollen

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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** Aliquot Nagel Catalog #740946 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 Add **□540** µL **Nagel Catalog #740506** to each warmed **⊒30 mL** ■ Lysis Buffer CF Macherey Nagel Catalog #740946 aliquot and invert gently to mix 4 **⊠** Lysis Buffer CF **Macherey**

Add ■560 µL Nagel Catalog #740946 +

⊠ Proteinase K Macherey

Nagel Catalog #740506 solution to each sample

Each sample will receive

Stysis Buffer CF Macherey

Nagel Catalog #740946

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5 Place in tissue lyser and run at **□10** Hz for **⊙00:01:00**

1m

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 8-80 °C

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

- 8 Add $\sim 100 \, \mu L$ 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**

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 8 -20 °C after this step

13 Add **□300 µL** (or equal volume)

⊠ Binding Buffer C4 **Macherey**

Nagel Catalog #740366.250

and $\blacksquare 300 \, \mu L$ (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

⊠ Binding Buffer C4 Macherey

■600 µL Nagel Catalog #740366.250

/EtOH



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⊠ Binding Buffer C4 **Macherey**

solution. Nagel Catalog #740366.250

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 **(3)1500 x g** for **(4)00:00:30**

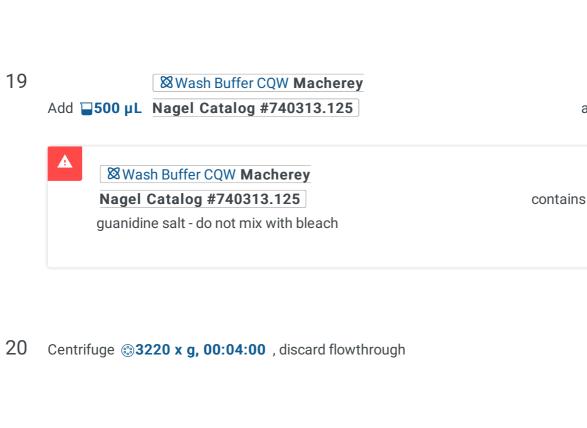
30s

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 gaspermeable foil
- 17 Centrifuge **3220 x g, 00:09:00**, discard flowthrough

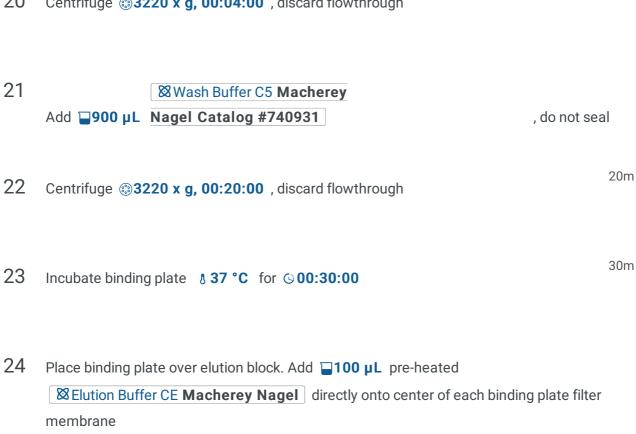
9m

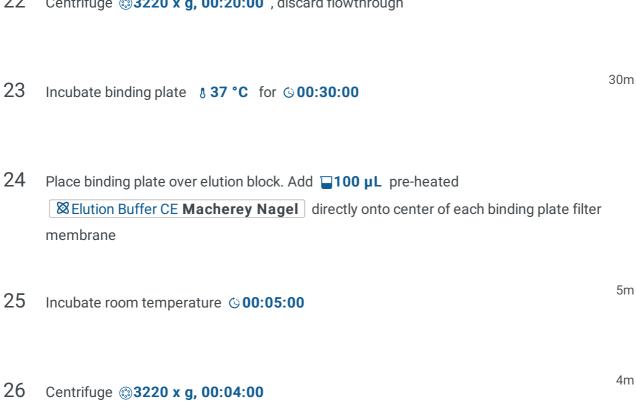
While centrifuging, aliquot out **□12 mL** per plate of **⊠Elution Buffer CE Macherey Nagel** and place in § **70 °C** water bath



and seal

4m







- 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)