dnature

• DETECT • DISCOVER •

Instruction Manual

Ver. 06.30.17

For Research Use Only

Genomic DNA Honey Kit

HDEB100 (100 Preparation Kit)

Advantages

Sample: 1 ml (approx. 1.4 g) of honey
Format: beadbeating tubes, genomic DNA spin columns
Time: within 60 minutes
Elution Volume: 50 µl
Kit Storage: dry at room temperature (15-25ºC)

Table of Contents

Introduction

The Genomic DNA Honey Kit was designed for isolation of genomic DNA from pollen in small honey samples. PCR inhibitors are removed with a unique inhibitor removal buffer before mechanical lysis is used to efficiently lyse the sample material. Ceramic bead tubes are then used to efficiently homogenize the sample material. Following sample lysis, the flow-through is then mixed with a binding buffer and the genomic DNA is bound by the GS Column. The column is then washed and the DNA is eluted with Elution Buffer. DNA phenol extraction or alcohol precipitation is not required and the entire procedure can be completed within 60 minutes. The purified genomic DNA is ready for use in qPCR and other downstream applications.

Quality Control

The quality of the Genomic DNA Honey Kit is tested on a lot-to-lot basis by isolating genomic DNA from 1 ml honey samples. Following the purification process, the purified genomic DNA is analyzed by qPCR.

Kit Components

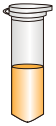
Component	HDEB004	HDEB100
PCR-Grade Water	4 ml	100 ml
Pol Buffer	2 ml	50 ml
GT Buffer	2 ml	50 ml
GBT Buffer	2 ml	30 ml
W1 Buffer	2 ml	45 ml
Wash Buffer[1] (Add Ethanol)	1 ml (4 ml)	25 ml (100 ml)
Proteinase K[2] (Add ddH_2O)	1 mg (0.1 ml)	11 mg x 2 (1.1 ml x 2)
Elution Buffer	1 ml	12 ml
Beadbeating Tubes Type D	4	100
GS Columns	4	100
2 ml Collection Tubes	8	200

[1]Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

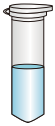
[2]Add ddH_2O (see the bottle label for volume) to prepare Proteinase K (vortex to dissolve and spin down) and store at 4ºC for short term use or in appropriate aliquots at -20ºC for longer term use (>1 month).

During the procedure, always wear a lab coat, disposable gloves, and protective goggles.

Quick Protocol Diagram

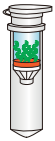


Pellet honey sample in a 2 ml microcentrifuge tube



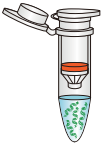
Pellet resuspension and inhibitor removal

Sample homogenization and lysis



DNA binding to membrane while contaminants remain suspended

Wash (removal of contaminants while DNA remains bound to membrane)



Elution of pure genomic DNA which is ready for subsequent reactions

Genomic DNA Honey Kit Protocol

Please read the entire instruction manual prior to starting the Protocol Procedure.

IMPORTANT BEFORE USE!

Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

Additional Requirements

- 2 ml and 1.5 ml flip top microcentrifuge tubes
- 1.5 ml flip top low-binding microcentrifuge tubes (e.g. Ayxgen maxymum recovery tube/Eppendorf Lo Bind microcentrifuge tube)
- Single use sticks or similar
- ≥95% ethanol
- Beadbeater
- Drybath/hotblock for 1.5/2 ml tubes (at 55-60ºC) or thermomixer

Protocol Procedure

1. PCR Inhibitor Removal

Label tubes appropriately for each individual sample, according to laboratory procedures. Transfer well-mixed, room temperature honey using a single use stick or similar means, up to the 1 ml graduation on a 2 ml microcentrifuge tube.

NOTE: Adding samples can be made easier by drawing a line with a thin marker across the 1 ml line on the tube. Alternatively, 1.4 g of honey may be weighed into a 2 ml tube.

Add **900 µl of PCR-Grade Water** to the honey in the 2 ml tube and vortex well. Incubate at 55ºC for 10 minutes. Invert the tube every 2-3 minutes, or use a thermomixer at 1,200 rpm.

NOTE: Samples should be homogeneous with no ‘swirls’ of honey evident when inverting tubes. If swirls remain after 10 minutes, incubate with further mixing for another 5 minutes or until no honey swirls are evident.

After heating, centrifuge at 15,000 x g for 5 minutes ensuring all tubes have hinges pointing to the outside of the centrifuge so that the pellet, even if not visible, can be located. Taking care not to disturb or remove the pellet on the hinge side of the tube (do not worry if pellet is not visible), remove the supernatant using a transfer pipette down the side of the tube that is opposite the hinge. Discard the transfer pipette containing the supernatant. Add **400 µl of Pol Buffer** to the 2 ml tube with the honey pellet and vortex briefly (3 seconds). Centrifuge at 15,000 x g for 5 minutes ensuring all tubes have hinges pointing outwards. Taking care not to disturb or remove the pellet on the hinge side of the tube (do not worry if pellet is not visible), remove the supernatant with a transfer pipette down the side of the tube that is opposite the hinge. Discard the transfer pipette containing the supernatant.

2. Sample Homogenization/Lysis

Add **380 µl of GT Buffer** to the pellet and gently resuspend by slowly pipetting up and down being sure to wash down any debris on the sides of the tube (3-5 times) and transfer the mixture to a pre-labelled **Beadbeating Tube**. Add **20 µl of Proteinase K.**

NOTE: Make sure to pipette onto the hinge side of tube where pellet should be located. Label Beadbeating Tubes on the top and sides in case these rub off during homogenisation. A premix of GT Buffer and Proteinase K may be made no earlier than 15 minutes prior to use. Allow extra for pipetting and add 400 µl of GT Buffer/Proteinase K per tube e.g. for 10 tubes add 4.04 ml of GT Buffer and 202 µl of Proteinase K to at tube, mix well and use among samples.

Homogenize in beadbeater instrument. Settings will depend on the instrument (e.g. if using a BioSpec instrument, beadbeat at maximum speed for 3 minutes). Incubate in a dryblock at 55ºC for 10 minutes, with mixing by inversion or brief vortexing every 2-3 minutes. Alternatively, a thermomixer may be used at 1,200 rpm.

Centrifuge the tubes for 1 minute at 15,000 x g.

3. DNA Binding

Transfer **200 µl of supernatant** from the **Beadbeating Tube** using a 1 ml pipette to a new labelled 1.5 ml microcentrifuge tube containing **200 µl of GBT Buffer**. Vortex and incubate in a dryblock (or thermomixer at 1,200 rpm) at 55ºC for 10 minutes, mixing every 2-3 minutes if a standard dryblock is used.

NOTE: During this time, transfer a volume equal to ~80 µl of Elution Buffer per sample together in a new 1.5-2.0 ml microcentrifuge tube and incubate in a dryblock at 60ºC for DNA Elution. For example, if 12 samples are being extracted, add ~960 µl of Elution Buffer to a tube and incubate.

After incubation, pulse centrifuge to collect contents at the bottom of the tube. Add **200 µl of absolute/95% ethanol** and mix by pipetting up and down 2-3 times. Place a **GS Column** in a **2 ml Collection Tube**. Transfer the entire mixture to the **GS Column**. Centrifuge at 15,000 x g for 1 minute. Discard the flow-through then place the **GS Column** back in the **2 ml Collection Tube**. NOTE: Alternatively, the GS column may be transferred to a fresh collection tube (not supplied in kit).

4. Wash

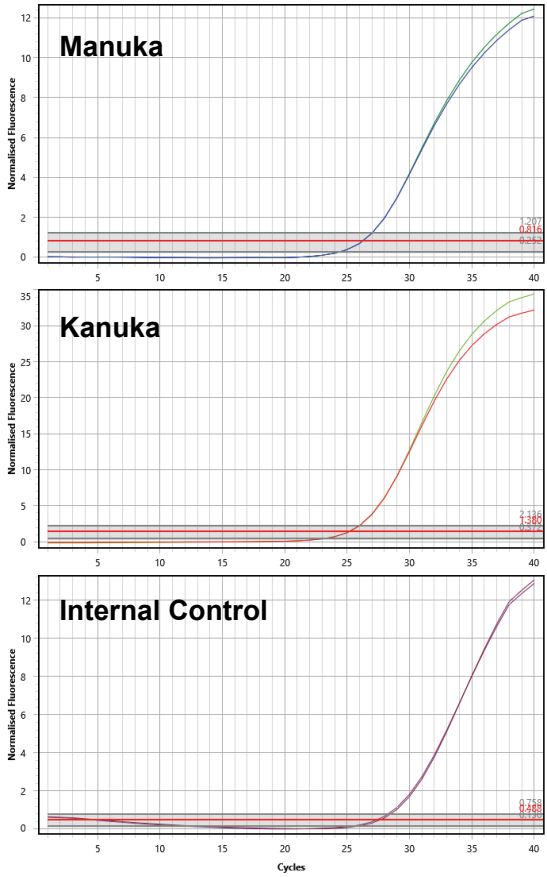
Add **400 µl of W1 Buffer** into the **GS Column** then centrifuge at 15,000 x g for 30 seconds. Discard the flow-through then place the **GS Column** back in the **2 ml Collection Tube**. Add **600 µl of Wash Buffer (make sure ethanol was added)** into the **GS Column**. Centrifuge at 15,000 x g for 30 seconds. Discard the flow-through then place the **GS Column** back in the **2 ml Collection Tube**. Add **200 µl of Wash Buffer** then centrifuge for 2 minutes at 15,000 x g to dry the column matrix.

5. Elution

NOTE: Care should be taken to ensure that the Elution Buffer covers the filter matrix and is not added to the side of the column. Slow dispensing to form a large 'drop' before adding buffer to the filter matrix can assist.

Transfer the dry **GS Column** to a new 1.5 ml microcentrifuge low DNA binding tube then add **50 µl of preheated Elution Buffer** into the CENTER of the column matrix. Incubate at 60ºC for 5 minutes to allow **Elution Buffer** to be completely absorbed. Centrifuge at 15,000 x g for 30 seconds at room temperature to elute the purified DNA. The purified honey DNA is now ready for qPCR and other downstream applications. DNA should be stored at 4ºC prior to testing for up to 3 days or at room temperature if testing within 1 hour of elution. Long term storage should be at -20ºC.

Genomic DNA Honey Kit qPCR Data



Figures 1, 2, 3. Genomic DNA from 1 ml of honey was extracted and qPCR'ed (in duplicate) on the Mic qPCR system (Biomolecular systems). Triplex qPCR (ManKan™ Honey real time PCR kit from dnature) was used to detect the presence of manuka (*L. scoparium*), kanuka (*K. ericoides*) and an internal control.


Beckman Coulter, Inc.
TA–15–15
max 15000


dnature
• DETECT • DISCOVER •