SOP

Extraction of DNA from Honey samples

QIAGEN GmbH
Postfach 10 64
D-40719 Hilden

1. SOP

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Attachments	DNeasy Tissue Handbook  C:\Documents and Settings\ullmanns\De:

2. Method

Formatiert: Nummerierung und Aufzählungszeichen

Material and instruments

For all protocols

- Pipets and pipet tips
- Vortexer
- Ethanol (96–100%)
- Microcentrifuge tubes (1.5 ml or 2 ml)
- Microcentrifuge with rotor for 1.5 ml and 2 ml tubes
- Thermomixer, shaking water bath, or rocking platform for heating at 56°C
- TissueLyser (QIAGEN GmbH)
- Tungsten Carbide Beads, 3 mm

2.2 Reagents

Ethanol (96-100%)
H_2O (MilliQ grade)
DNeasy Blood & Tissue Kit (QIAGEN GmbH)

DNeasy Tissue Kit	
	(50)
	(250)
Catalog no.	

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	69504
	69506
Number of preps	
	50
	250
DNeasy Mini Spin Columns (colorless) in 2 ml Collection Tubes	
	50
	250
Collection Tubes (2 ml)	
	100
	500
Buffer ATL	
	10 ml
	50 ml
Buffer AL*	
	12 ml
	54 ml
Buffer AW1 (concentrate)*†	
	19 ml
	95 ml
Buffer AW2 (concentrate)†‡	
	13 ml
	66 ml
Buffer AE	

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	22 ml
	2 x 60 ml
Proteinase K	
	1.25 ml
	6 ml
Handbook	
	1
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* Contains a chaotropic salt. Not compatible with disinfecting agents containing bleach. See page 8 for safety information.

† Buffer AW1 and Buffer AW2 are supplied as concentrates. Add ethanol (96–100%) according to the bottle label before use to obtain a working solution.

‡ Contains sodium azide as a preservative.

2.3 Protocol

Dissolving and disruption of honey

1. **Weigh up to 10 g honey in a 50 ml tube.**
2. **Add mqH_2O to a final volume of 45 ml.**
3. **Incubate at 65°C in a water bath with shaking for 30 min or until the honey has dissolved.**
4. **Centrifuge sample at 2400 x g for 30 min at RT.**
   If possible centrifuge at a higher speed, up to 5000 x g is recommended, the time can be reduced to 15 min.
5. **Discard supernatant, resuspend pellet in the leftover of the supernatant (approx. 200µl)**
   This pellet contains pollen.
6. **Transfer 200 µl into a 2ml tube, together with a 3 mm Tungsten Carbide Bead.**
7. **Place the tubes into the TissueLyser Adapter Set and fix into the clamps of the TissueLyser. Immediately grind the samples for 2 min at 30 Hz.**
8. **Add 50 µl of Buffer ATL together with 25 µl proteinase K (20mg/µl)**

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9. **Incubate for 30 min at 56°C.**
   If possible use a Thermomixer at 1000 rpm.

10. **Continue with the Isolation and Purification method.**

Dissolving and disruption of Pollen

1. **Weigh up to 50 mg pollen in a 2 ml tube together with a 3 mm Tungsten Carbide beads.**

2. **Place the tubes into the TissueLyser Adapter Set and fix into the clamps of the TissueLyser. Immediately grind the samples for 2 min at 30 Hz.**

3. **Add 200 µl of Buffer ATL together with 25 µl proteinase K (20mg/µl)**

4. **Incubate for 30 min at 56°C.**
   If possible use a Thermomixer at 1000 rpm.

5. **Continue with the Isolation and Purification method.**

Isolation and Purification method using the DNeasy Tissue Kit

1. **Ensure that the sample tube containing the pollen DNA is prepared as described above.**

2. **optional: Add 10 µl of RNAse (40mg/ml), mix by vortexing, and incubate for 2 min at room temperature.**

3. **Add 200 µl Buffer AL to the sample, mix thoroughly by vortexing and incubate at 70°C for 10 min.**

4. **Add 200 µl ethanol (96-100%) to the samle, and mix thoroughly by vortexing.**

5. **Pipet the mixture from step 3 into the DNeasy Mini column placed in a 2 ml collection tube. Centrifuge at ≥6000 x g (8000rpm) for 1 min. Discard flow-through and collection tube.**

6. **Place the DNeasy Mini spin column in a new 2 ml collection tube, add 500 µl Buffer AW1, and centrifuge for 1 min at ≥6000 x g (8000rpm). Discard flow-through and collection tube.**

7. **Place the DNeasy Mini spin column in a 2 ml collection tube, add 500 µl Buffer AW2, and centrifuge for 3 min at 20,000 x (14,000 rpm) to dry the DNeasy membrane. Discard flow-through and collection tube.**

8. **Place the DNeasy Mini spin column in a clean 1.5 ml or 2 ml microcentrifuge tube, and pipet 100 µl Buffer AE directly onto the DNeasy membrane. Incubate at room temperature for 1 min, and then centrifuge for 1 min at at ≥6000 x g (8000rpm) to elute.**

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3. Analysis

The DNA can be used for PCR reaction, Liquichip experiments and other down stream analysis.