Promega

Product Application

DNA Extraction from Honey using the Maxwell® RSC System

Isolate high-quality, amplifiable DNA from honey using the Maxwell® RSC System.

Kit: Maxwell® RSC PureFood GMO and Authentication Kit (Cat.# AS1600)

Analyses: Absorbance, QuantiFluor® quantification, qPCR

Sample Type: Honey

Input: 40ml

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

For further information, see Technical Manual TM473, available at: **www.promega.com/protocols**

or contact Technical Services at: techserv@promega.com

Materials Required:

- Maxwell® RSC Instrument (Cat.# AS4500)
- Maxwell® RSC PureFood GMO and Authentication Kit (Cat.# AS1600)
- Centrifuge (50ml conical tube capacity)
- homogenizer – bead beater (MP Biomedical FastPrep-24™)
- MP Biomedical Lysing Matrix E, 2ml tube (MP Biomedical, Cat. #116914050)

Protocol:

1. Per sample, add 10ml of honey to each of four 50ml conical tubes and dilute with 40ml of water each. Invert until mixed.
2. Spin the samples at 4000 × *g* for 10 minutes. Remove and discard the supernatant.
3. Add 2ml of water to the pellet and vortex for 5 seconds.
4. Pool together the 4 samples into a 50ml conical tube and add water to 40ml.
5. Spin the samples at 4000 × *g* for 10 minutes. Remove and discard the supernatant.
6. Suspend the pellet in 1ml CTAB buffer, and place sample into Lysing Matrix E tube. Homogenize sample using a bead beater (FastPrep-24™ – 40secs at 6M/s).
7. Add 20µl of RNase A and 40µl of Proteinase K Solution. Vortex to mix.
8. Place in a heat block at 65°C for 30 minutes.
9. Prepare RSC cartridges and elution tubes (100µl) as described in the technical manual (TM473).
10. Spin the samples at 16,000 × *g* for 10 minutes.
11. Transfer 300µl of clear lysate into well #1 of the reagent cartridge.
12. Add 300µl of Lysis Buffer to well #1 of each cartridge.
13. Run the Maxwell® RSC Instrument as described in the technical manual.

Results:

The above protocol was tested using 40ml honey per DNA extraction (n=3).

Table 1. Honey DNA yield (ng) based on quantitation using the QuantiFluor® ONE dsDNA System (Cat.# E4871). Absorbance ratios based on NanoDrop®-One spectrophotometer. Mean ± STD of n=3.

Honey (40ml)		
	mean	STD
Yield (ng)	363.5	3.6
A260/A280	2.02	0.03
A260/A230	2.11	0.09

Table 2. Analysis of purified DNA using GoTaq® qPCR Master Mix (Cat.# A6001) and Plant Universal Primers (1) using 5µl of DNA eluate per 25µl reaction. DNA samples added to the qPCR reaction were either neat, diluted 10-fold or diluted 100-fold. Efficiency was determined using the Cq values from this serial dilution. Mean ± STD of n=3.

qPCR using Plant universal primers							
Cq (Neat)		Cq (1/10 dil)		Cq (10/100 dil)		Efficiency	
mean	STD	mean	STD	mean	STD	mean	STD
21.5	0.2	24.6	0.0	28.0	0.1	103.6	2.1

Reference:

1. Wang, J. *et al.* (2011) Universal endogenous gene controls for bisulphite conversion in analysis of plant DNA methylation. *Plant Methods* **7**, 39.