Promega

Product Application

DNA Purification from Carrot Seed using the Maxwell® RSC System

High-quality DNA from carrot seed was purified using the Maxwell® RSC PureFood GMO and Authentication Kit.

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

Analyses: NanoDrop™ ONE Spectrophotometer and Quantus™ Fluorometer quantitation, GoTaq® qPCR MasterMix amplification

Sample Type(s): Carrot seed homogenates in PBS

Input: 1ml at 100mg/ml

Materials Required:

- Maxwell® RSC Instrument (Cat.# AS4500)
- Maxwell® RSC PureFood GMO and Authentication Kit (Cat.# AS1600)

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**

Protocol:

1. Centrifuge samples for 10 minutes at 7500rpm and remove supernatant.
2. For each sample, prepare preprocessing solution by mixing 1ml of CTAB with 20µl of RNase A and 40µl of Proteinase K (PK) Solution per sample. Resuspend samples in 1ml of this solution.
3. Vortex until sample is completely resuspended.
4. Incubate samples in a heat block for 30 minutes at 65°C with shaking at 1000rpm.
5. During incubation, prepare RSC cartridges as described in the Technical Manual TM473. Add 100µl of Elution Buffer into the Elution Tubes.
6. Invert sample tubes thoroughly and centrifuge at room temperature for 10 minutes at maximum speed to remove cellular debris.
7. Add 300µl of Lysis Buffer, as well as 300µl of cleared lysate, into well #1 of the cartridge. Run the PureFood GMO and Authentication method on the Maxwell® RSC Instrument.

Results:

Quantus (ng/µl)	NanoDrop (ng/µl)	A260/280	A260/230
5.26 ± 1.05	50.84 ± 3.73	1.91 ± 0.02	1.9 ± 0.22

Figure 1. DNA concentration and purity obtained from carrot seed samples using the Maxwell® RSC PureFood GMO and Authentication Kit. Assessed by NanoDrop® Spectrophotometer and Quantus™ Fluorometer (Cat.# E6150) using QuantiFluor® ONE dsDNA System (Cat.# E4870). N=9.

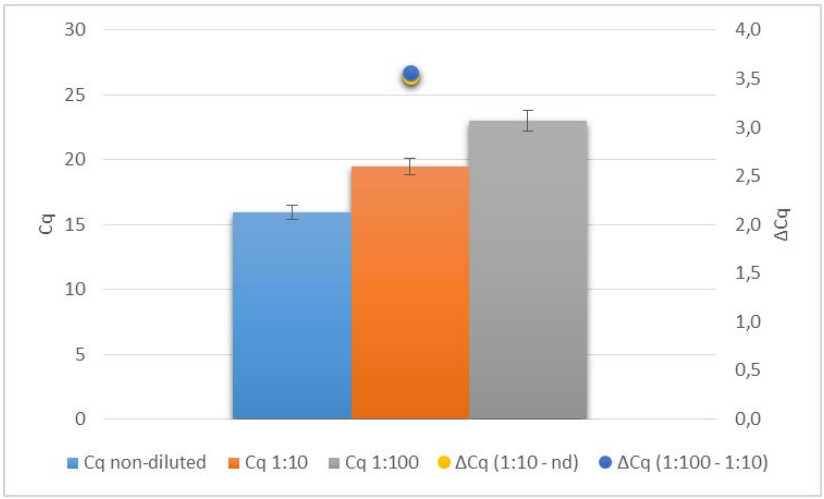

Figure 2. qPCR amplification of DNA purified from carrot seed using the Maxwell® RSC PureFood GMO and Authentication Kit. Cq and ΔCq values shown are for 2µl of DNA amplified using the GoTaq® qPCR Master Mix (Cat.# A6001) and universal plant primers (1) in a final volume of 20µl. A ΔCq value of 3.3 reflects the total absence of qPCR inhibitors. N=9.

Reference:

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