Promega

Product Application

DNA Extraction from Chocolate using the Maxwell® RSC System

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

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

Analyses: Absorbance, QuantiFluor® quantification, qPCR

Sample Type: Chocolate

Input: 100mg

Materials Required:

- Maxwell® RSC Instrument (Cat.# AS4500)
- Maxwell® RSC PureFood GMO and Authentication kit (Cat.#AS1600)
- heat block (Eppendorf Thermomixer)
- OneStep™ PCR inhibitor removal kit (Zymo Research Cat.# D6030)

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. In a 1.5ml microcentrifuge tube, place 100mg of chocolate.
2. Add 1ml CTAB buffer, 20µl RNase A and 40µl Proteinase K Solution.
3. Vortex for 15 seconds to mix.
4. Place in a heat block at 65°C for 30 minutes (if possible use a shaking heat block with constant shaking).
5. Prepare RSC cartridges and elution tubes (100µl) as described in technical manual (TM473).
6. Spin the samples at 16,000 × *g* for 10 minutes.
7. Transfer 300µl of clear lysate into well #1 of the reagent cartridge.
8. Add 300µl of Lysis Buffer to well #1 of each cartridge.
9. Run the Maxwell® RSC Instrument as described in the technical manual.
10. After the method is complete, add the eluate to a prepared Zymo-Spin™ IV-HRC column.
11. Spin at 8,000 *x g* for 1 minute. Discard spin column.

Results:

The above protocol was tested using 100mg of chocolate* per DNA extraction (n=3). The Zymo Research OneStep™ PCR inhibitor removal kit was used to remove qPCR inhibitors.

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

	Chocolate (100mg)	
	mean	STD
Yield (ng)	116.2	6.5
A260/A280	1.39	0.03
A260/A230	0.60	0.04

Table 2. Analysis of purified DNA using GoTaq® qPCR Master Mix (Cat.# A6001) with Plant Universal Primers (1) and Animal Universal Primers (2) using 5µl 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 and Animal universal primers							
	Cq (Neat)		Cq (1/10 dil)		Cq (10/100 dil)		Efficiency	
	mean	STD	mean	STD	mean	STD	mean	STD
Plant primers	25.36	0.10	28.64	0.19	32.53	0.57	91.1	2.5
Animal primers	30.87	0.16	34.31	0.66	37.13	0.96	108.0	1.1

* Perugina 51% Cacao – sugar, chocolate, cocoa butter and 1% sunflower lecithin; may contain traces of nuts and milk.

References:

1. Wang, J. *et al*. (2011) Universal endogenous gene controls for bisulfite conversion in analysis of plant DNA methylation. *Plant Methods* **7**, 39.
2. Sawyer, J. *et al*. (2003) Real-time PCR for quantitative meat species testing. *Food Control* **14**, 579–83.