Promega

Product Application

DNA Extraction from Fish Sticks using the Maxwell® RSC System

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

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

Analyses: Absorbance, QuantiFluor® quantification, qPCR

Sample Type: Breaded frozen fish sticks (wheat breading, Pollock fish)

Input: 2g

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 compatible with 15ml conical tubes
- heat block compatible with 15ml conical tubes (e.g., Eppendorf Thermomixer)

Protocol:

1. In a 15ml conical tube, place 2g of fish stick.
2. Add 10ml of CTAB buffer, 200µl of RNase A and 400µl of 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 the technical manual (TM473).
6. Spin the samples at 4,000 × *g* for 10 minutes.
7. Transfer 1ml of clear lysate to a 1.5ml microcentrifuge tube.
8. Spin the samples at 16,000 × *g* for 2 minutes.
9. Transfer 300µl of clear lysate into well #1 of the reagent cartridge.
10. Add 300µl of Lysis Buffer to well #1 of each cartridge.
11. Run the Maxwell® RSC Instrument as described in the technical manual.

Results:

The above protocol was tested using 2g of fish stick per DNA extraction (n=3).

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

	Fish stick (2g)	
	mean	STD
Yield (µg)	18.2	5.3
A260/A280	2.01	0.03
A260/A230	2.20	0.02

Table 2. Analysis of purified DNA using GoTaq® qPCR Master Mix (Cat.# A6001) with Plant Universal Primers (1) and Fish Universal Primers (2) using 5µl of DNA sample per 25µl reaction. DNA samples added to the qPCR reaction were first diluted to 10ng/µl and then serially diluted 10-fold and 100-fold. Efficiency was determined using the Ct values from this serial dilution. Mean ± STD of n=3.

	qPCR using Plant and Fish universal primers							
	Cq (50ng)		Cq (5ng)		Cq (0.5ng)		Efficiency	
	mean	STD	mean	STD	mean	STD	mean	STD
Plant primers	21.39	0.35	24.55	0.47	28.04	0.43	99.9	2.9
Fish primers	20.70	0.34	24.01	0.24	27.33	0.29	100.4	1.4

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. Garrett, S. and Dooley, J. (2005) Determination of PCR-RFLP profiles for fish species using the Agilent 2100 Bioanalyzer Application Note.