Promega

Product Application

DNA Purification from Processed Meat using the Maxwell® RSC System

High-quality DNA suitable for downstream applications was purified from processed meat samples using the Maxwell® RSC PureFood GMO and Authentication Kit.

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

Analyses: NanoVue™ spectrophotometer and Quantus™ Fluorometer quantitation, qPCR amplification

Sample Type(s): Chicken sausage, pork gelatin, beef ravioli, breaded fish

Input: 50mg

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)

Protocol:

Six samples were analyzed for each sample type; 3 of them were incubated for 90 minutes, and 3 of them were incubated for 30 minutes. Extractions were performed according to the following protocol:

1. Homogenize samples using a mortar and pestle precooled at –80 °C and/or a mixer.
2. Weigh 50mg of sample.
3. Add 1ml of CTAB, 40µl of Proteinase K and 20µl of RNase A to each tube and vortex vigorously.
4. Incubate samples at 65°C, 600rpm, for 90 or 30 minutes, depending on the sample group.
5. Vortex and invert sample tubes; then centrifuge at high speed for 10 minutes.
6. Add 100µl of elution buffer to elution tubes.
7. Add 300µl of lysis buffer and 300µl of sample into the first well of the Maxwell® cartridge.
8. Run the Maxwell® RSC Instrument using the Maxwell® RSC PureFood GMO and Authentication Kit protocol.

Results:

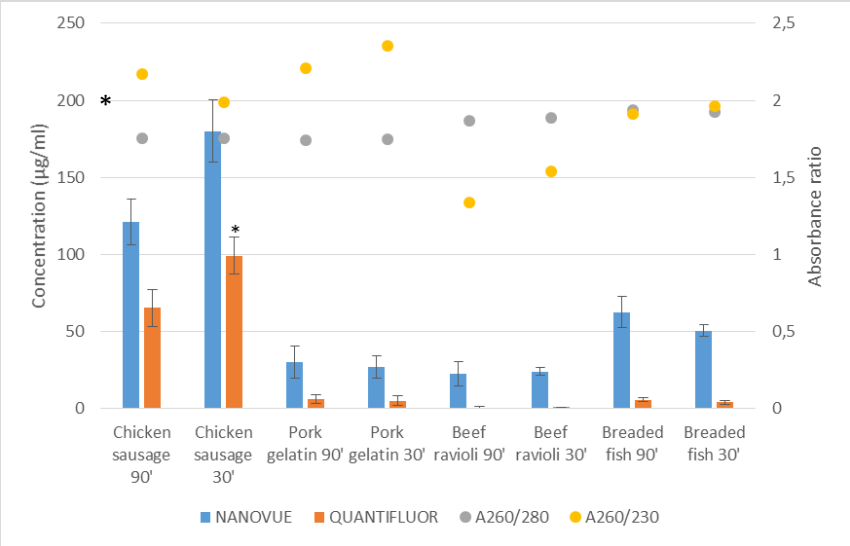

Figure 1. DNA concentration and purity obtained with the Maxwell® RSC PureFood GMO and Authentication Kit. Assessed by NanoVue™ spectrophotometer and Quantus™ Fluorometer (Cat.# E6150). N=3.

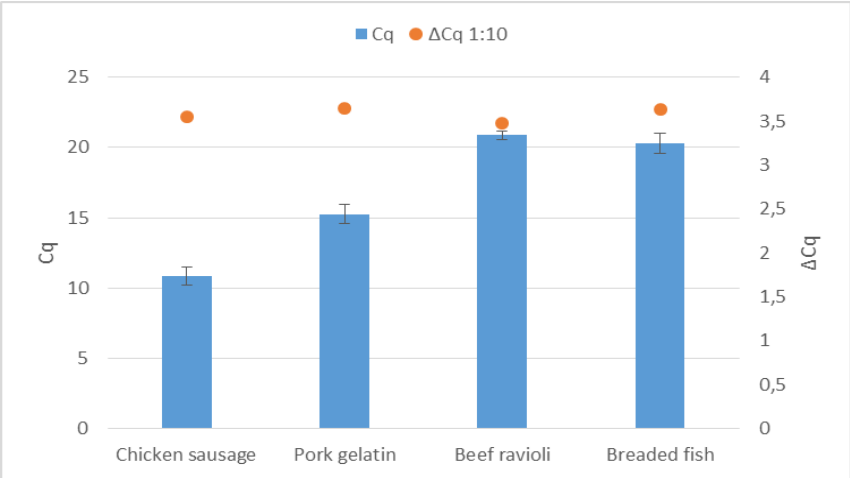

Figure 2. qPCR amplification and inhibition test data. Cq and ΔCq values for 2µl of eluted DNA amplified using the GoTaq® qPCR Master Mix (Cat.# A6001) in a final volume of 20µl. Universal animal primers M13U12S (1) were used for chicken sausage and breaded fish samples amplification. Pork gelatin and beef ravioli samples were amplified using specific pork and beef primers (2), respectively. ΔCq values suggest that no qPCR inhibition occurred. N=3.

References:

1. Yang, L. *et al.* (2014) Species identification through mitochondrial rRNA genetic analysis. *Scientific Reports* **4**, 4089.
2. López-Andreo, M. *et al*. (2005) Identification and quantitation of species in complex DNA mixtures by real-time polymerase chain reaction. *Anal. Biochem*. **339**, 73–82.