Promega

Product Application

Manual DNA extraction from food samples for GMO detection

Manual DNA extraction from food samples using ReliaPrep™ Blood gDNA Miniprep System for GMO detection.

Kit: *ReliaPrep™ Blood gDNA Miniprep System* (Cat. #A5081)

Analyses: Quantitation by absorbance and with fluorescent dye. Probe-based qPCR amplification.

Sample Type(s): Pretzels (origin USA and Europe), corn chips (origin USA and Europe), ground corn (Europe) and Maize GMO Standard.

Input: 100mg of ground samples

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.

Further information available at: www.promega.com/protocols

or by e-mailing technical services at techserv@promega.com

Materials Required:

- ReliaPrep™ Blood gDNA Miniprep System (Cat. #A5081)
- CTAB Buffer (Cat. #MC1411)
- RNase A Solution (Cat. #A7973)
- Proteinase K (PK) Solution (Cat. #A505C)
- Elution Buffer (Cat. #A8281)
- Isopropanol 100%.
- Maize GMO Standard (Sigma-Aldrich ref. ERM-BF412F)
- Frozen mortar and pestle
- Heat block
- Microcentrifuge

Protocol:

1. Grind sample with a frozen mortar and pestle.
2. Add 1ml of CTAB buffer, 20µl of RNase A Solution and 40µl of Proteinase K (PK) Solution to each tube containing 100mg of ground sample. Tap, invert and vortex tubes until the sample is suspended.
3. Place in a heat block at 65°C for 30 min with shaking at 600rpm. After incubation, vortex tubes with lysate to mix thoroughly.
4. Centrifuge 10 min at 13,400 rpm to separate any solid or oils.
5. Transfer 300µl of cleared lysate to a clean 1.5ml microtube; avoid pipetting any solid or oils.
6. Add 300µl of CLD Buffer (Cell Lysis Buffer). Add 600µl of 100% of Isopropanol. Mix by inversion.
7. Load 600µl of sample to the ReliaPrep™ Binding Column placed in a collection tube. Centrifuge for 1 min at maximum speed. Discard the flow through. Load the rest of the sample to the ReliaPrep™ Binding Column. Spin for 1 min. Place the ReliaPrep™ Binding Column into a new collection tube.
8. Add 500µl of Column Wash Buffer (CWB). Spin 2 min at maximum speed. Discard the flow through. Repeat step 7 twice, for a total of three washes.

9. Place the ReliaPrep™ Binding Column in a labeled elution tube. Add 100µl of Elution Buffer to the ReliaPrep™ Binding Column and spin 1 min at maximum speed. Discard the ReliaPrep™ Binding Column and save eluate.

Results:

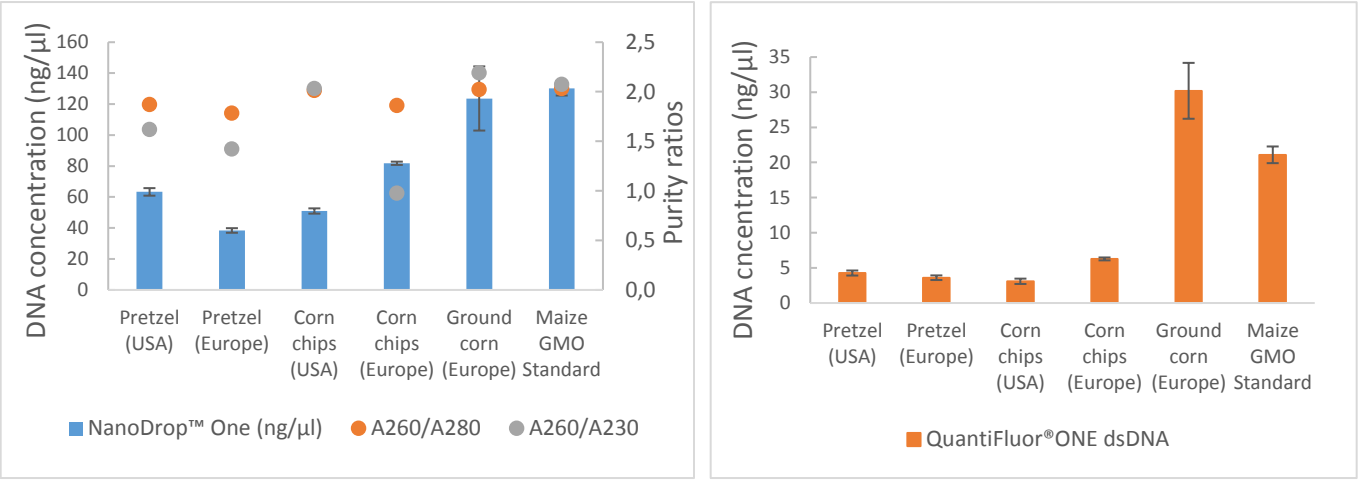

Figure 1. DNA concentration and purity ratios of DNA extracted from 100mg of food samples with European or USA origin using ReliaPrep™ Blood gDNA Miniprep System (Cat. #A5081). DNA concentration and purity ratios were assessed by absorbance on the NanoDrop™ One Spectrophotometer (left) and with the QuantiFluor® ONE dsDNA System (Cat. #E4870) (right). Maize GMO Standard (Sigma-Aldrich ref. ERM-BF412F).

Table 1. qPCR amplification results for DNA extracted from 100mg of food samples with European or USA origin containing or not GMO, using the ReliaPrep™ Blood gDNA Miniprep System. Amplification of 2µl of DNA eluates amplified using GoTaq® Probe qPCR Master Mix (Cat. #A6102) and P35S specific primers and probe[2] in a final volume of 20µl (N=3). Maize GMO Standard (Sigma-Aldrich ref. ERM-BF412F).
**Different regulatory affair about GMO presence in food.

Sample	DNA amplifiable with universal plant primers[1]	DNA amplifiable with GMO specific primers[2]	Expected presence of GMO **
Pretzel (USA)	+	+	+
Pretzel (Europe)	+	-	-
Corn chip (USA)	+	+	+
Corn chip (Europe)	+	-	-
Ground corn (Europe)	+	-	-
Maize GMO Standard	+	+	+

Reference:

1. Wang et al.: *Universal endogenous gene controls for bisulphite conversion in analysis of plant DNA methylation.* Plan Methods 2011 7:39.
2. Specific probe-primers P35S for GMO detection. Designed by Leta Steffen, for qPCR training.