Promega

# Product Application

## Manual DNA Extraction from Food Samples for GMO Detection

*Manual DNA purification from food samples using the ReliaPrep™ Blood gDNA Miniprep System for GMO detection followed by amplification using GoTaq® Probe qPCR Master Mix.*

**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.

For further information, see: www.promega.com/protocols

or e-mail technical services at: techserv@promega.com

**Materials Required:**

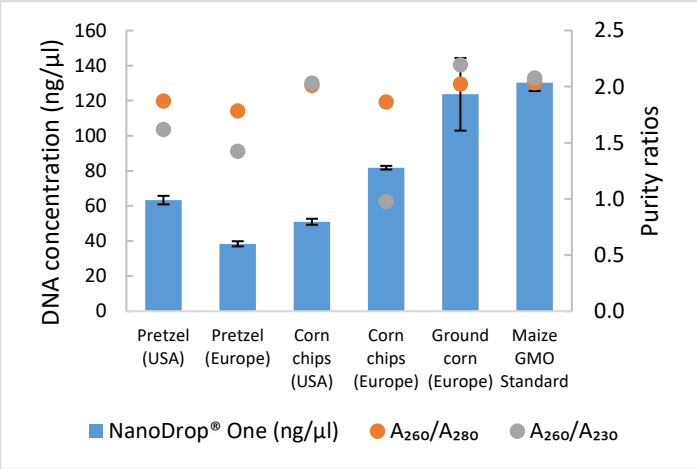
- ReliaPrep™ Blood gDNA Miniprep System (Cat.# A5081)
- CTAB Buffer (Cat.# MC1411)
- RNase A Solution (Cat.# A7973)
- Proteinase K (PK) Solution (Part# A505C)
- Elution Buffer (Cat.# A8281)
- 100% isopropanol
- Maize GMO Standard (Sigma-Aldrich® Cat.# ERM-BF412F)
- GoTaq® Probe qPCR Master Mix (Cat.# A6102)
- QuantiFluor® ONE dsDNA System (Cat.# E4871)
- 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 minutes with shaking at 600rpm. After incubation, vortex tubes with lysate to mix thoroughly.
4. Centrifuge for 10 minutes at 13,400 rpm to separate any solids or oils.
5. Transfer 300µl of cleared lysate to a clean 1.5ml microcentrifuge tube; avoid pipetting any solids or oils.
6. Add 300µl of CLD Buffer (Cell Lysis Buffer). Add 600µl of 100% isopropanol. Mix by inversion.
7. Load 600µl of sample to the ReliaPrep™ Binding Column placed in a collection tube. Centrifuge for 1 minute at maximum speed. Discard the flowthrough. Load the rest of the sample onto the ReliaPrep™ Binding Column. Centrifuge for 1 minute. Place the ReliaPrep™ Binding Column into a new collection tube.

8. Add 500µl of Column Wash Buffer (CWB). Centrifuge for 2 minutes at maximum speed. Discard the flowthrough. 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 centrifuge for 1 minute at maximum speed. Discard the ReliaPrep™ Binding Column and save eluate.

**Results:**



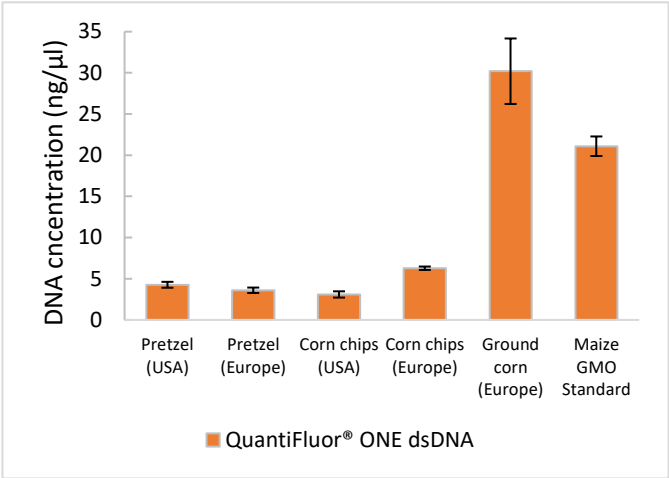




**Figure 1. DNA concentration and purity ratios of DNA extracted from 100mg of food samples of European or USA origin using ReliaPrep™ Blood gDNA Miniprep System.** (Cat.# A5081). DNA concentration and purity ratios were assessed by absorbance on the NanoDrop® One Spectrophotometer (left) and with the QuantiFluor® ONE dsDNA System (Cat.# E4870) (right). Maize GMO Standard (Sigma-Aldrich® Cat.# ERM-BF412F) was used as a positive control for the presence of GMO. Error bars indicate standard deviation (N=3).

**Table 1. qPCR amplification results for DNA extracted from 100mg of food samples from Europe or USA, with or without GMO, using the ReliaPrep™ Blood gDNA Miniprep System**. Two microliters of DNA eluates were amplified using the 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® Cat.# ERM-BF412F) was used as a positive control for the presence of GMO.

| 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 | + | + | + |

*The USA has no federal standards for what defines a GMO-positive or -negative food sample. In the EU, a positive is defined as "a maximum of 0.9% of genetically modified plant material per ingredient (e.g. grain, flour, syrup)." (3)

**References:**


1. Wang, J. *et al.* (2011) Universal endogenous gene controls for bisulphite conversion in analysis of plant DNA methylation. *Plant Methods* **7**:39.
2. Specific probe-primers P35S for GMO detection. Designed by Leta Steffen (Promega Corporation) for qPCR training.
3. EFSA Panel on Genetically Modified Organisms *et al.* (2017) Scientific Opinion on guidance for the risk assessment of the presence at low level of genetically modified plant material in imported food and feed under Regulation (EC) No 1829/2003. *EFSA Journal* **15**:5048–67.