TOOLS GMO detection Kit

For isolation of DNA and PCR amplification of genetically modified organisms

Contents

Introduction

TOOLS GMO detection kit is designed for analyzing genetically modified organism (GMO) crops, such as wheat, corn, rice, cotton, and soy. This kit contains DNA extraction and PCR reagents. Simple centrifugation processing and phenol/chloroform completely remove contaminants, RNA, and enzyme inhibitors such as proteins and divalent cations. The high-quality DNA can be used for subsequent PCR detection using PCR Taq and buffer provided by this kit.

Important Notes

1. Avoid repeated thawing of samples because this leads to smaller fragments of DNA and lower yield.
2. Using fresh crops (leaves or seeds) to obtain higher DNA yields and quality.
3. Every centrifugation step should be at room temperature.
4. Add β-mercaptoethanol into PBL Buffer before use (final concentration: 0.1%, V/V).

Kit Contents

Contents	KGT-BB02 200 preps
PBL Buffer	160 mL
TE Buffer	2 × 15 mL
RNase A (100 mg/ml)	1.25 mL
2x PCR buffer	4 mL
DNA polymerase (2.5 U/µl)	400 U

Storage

PBL Buffer, TE Buffer, and RNase A should be stored in a dry place at room temperature (15–25 °C). The 2× PCR buffer and DNA polymerase should be stored at −20 °C. This kit is stable for 12 months under the aforementioned storage conditions.

Protocol

1. Place the tissue sample (100 mg wet weight or 20 mg lyophilized) into a mortar, add liquid nitrogen to the mortar, and grind the sample thoroughly.

Note: Because of plant diversity, the starting amount of plant tissue should be adjusted according to the species and part of the crop.

2. Transfer the processed sample to 700 µL of PBL Buffer (65 °C) immediately (preheat PBL Buffer and add β-mercaptoethanol according to Important note 4). Mix by inverting and incubate the tube at 65 °C for 20 min (inverting the tube several times during incubation).
3. Collect the residual drops and liquid by short centrifugation. Add 6 µL of RNase A (100 mg/mL), vortex vigorously for 1 min, and let the sample stand at room temperature for 10 min.
4. Add an equivalent volume of phenol:chloroform:isoamyl alcohol (25:24:1) and then mix on a vortex mixer. Centrifuge for 5 min at 12,000 rpm (~13,400 ×g).

Note: Do one more extraction with phenol:chloroform (1:1) if the crop contains a high polyphenol or starch content.

5. Transfer the upper layer aqua phase to a new tube, and add an equivalent volume of isopropanol. Mix well.
6. Centrifuge for 5 min at 12,000 rpm (~13,400 ×g).
7. Discard the supernatant, add 500 µL of 70% ethanol, and mix on a vortex mixer.
8. Centrifuge for 5 min at 12,000 rpm (~13,400 ×g).
9. Repeat Steps 7 and 8. Open the lid, and incubate at room temperature (15–25 °C) for 5–10 min to dry the precipitate and remove the residual alcohol.

Note: Ensure that no ethanol is carried over. Residual ethanol may interfere with downstream reactions (enzyme digestion reaction, PCR, etc.).

10. Pipette 100 µL of TE Buffer, and incubate at 65 °C for 10–60 min in a water bath. Turn the tube upside down during the incubation so as to dissolve purified DNA in TE Buffer.

11. Prepare the PCR reaction according to the following table (example):

Contents	Volume
Template	< 500 ng
Primer 1(10 μM)	0.4 μL
Primer 2(10 μM)	0.4 μL
GMO DNA Polymerase (2.5 U/μl)	1U
2×GMO PCR Buffer	10 μL
ddH2O	Add to make 20 μL

12. PCR cycle set-up (adjust the annealing temperature according to primer design):

94℃ 5 min

94℃ 15 sec
60℃ 20 sec
72℃ 20 sec
} 35 cycles

72℃ 5 min

13. Load 5 μL of PCR products on agarose gel for detecting.