GMO Crop Extraction & Amplification Kit (Part A)

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Cat. No. 4992890

Kit Contents

Contents	4992890 (200 preps)
Buffer PL	160 ml
RNase A (100 mg/ml)	1.25 ml
Buffer TE	2×15 ml
Handbook	1

Storage Conditions

The kit can be stored for 12 months under dry conditions at room temperature (15-25°C).

Introduction

This product is specially developed for the PCR detection of GMO crop. The kit includes two parts: A and B. Part A of the kit provides reagents for extracting GMO crop genomic DNA, while Part B provides reagents for PCR detection of GMO crop DNA.

The unique lysis buffer contained in Part A of the kit can specifically and fully lyse the tissues of five main crops—wheat, corn, rice, cotton and soybean to release nucleic acid, protein and other related components. Subsequent RNA, protein, metal ions and other impurities can be removed to the maximum extent through specific RNase and phenol/chloroform extraction to obtain genomic DNA with good purity and high yield for subsequent PCR detection.

Features

Wide applicability: High-quality genomic DNA can be extracted from five major GMO crops.

Easy and fast: The extraction of GMO crop genomic DNA can be completed within 2 hours with no need for a large-scale freezing centrifuge. The experiment has low requirements on instruments and equipment, thus being suitable for research institutions at all levels to carry out rapid genomic DNA extraction on GMO crops.

Important Notes Before Starting

1. Avoid repeated freezing and thawing of the sample, otherwise the extracted DNA fragments will be smaller and the extraction yield will be decreased.
2. It is suggested to use fresh leaves for extraction to acheive optimal yield and purity of genomic DNA.
3. All centrifugation steps are performed at room temperature using a benchtop centrifuge.
4. Before using Buffer PL, please add mercaptoethanol to the final concentration of 0.1%(V/V).

Protocol

1. Take about 100 mg of fresh plant tissue or about 30 mg of dry weight tissue, and grind with liquid nitrogen.
2. Quickly transfer the ground powder to a centrifuge tube with 700 µl of 65°C preheated Buffer **PL (add mercaptoethanol to the preheated Buffer PL to make its final concentration 0.1% before the experiment).** After quickly inverting and mixing, place the centrifuge tube in a 65°C water bath for 20 min. During the water bath, invert the centrifuge tube to mix the samples several times.
3. Take out the centrifuge tube, and gently flick the tube to collect the liquid drops on the tube cover and tube wall to the bottom. Add 6 µl of RNase A (100 mg/mL), mix well, and incubate at room temperature for 10 min.
4. Add equal volume of phenol: chloroform: isoamyl alcohol (25:24:1), mix thoroughly and centrifuge at 12,000 rpm (~13,400×g) for 5 min.

   Note: For plant tissues rich in polyphenol or starch, the equal volume of phenol: chloroform (1:1) can be used for a second time extraction.

5. Carefully transfer the upper aqueous phase obtained in the previous step into a new centrifuge tube, add equal volume of isopropanol, and mix thoroughly.
6. Centrifuge at 12,000 rpm(~13,400×g) for 5 min.
7. Discard the supernatant, add 500 µl 70% ethanol and mix well.
8. Centrifuge at 12,000 rpm(~13,400×g) for 5 min.
9. Repeat steps 7 and 8.
10. Invert the centrifuge tube on clean absorbent paper until the ethanol volatilizes completely. Make sure the precipitation remain in the centrifuge tube.

    Note: The residue of ethanol will affect the subsequent enzyme reaction (enzyme digestion, PCR, etc.). However, over-drying of DNA precipitation should be avoided, for it will be difficult to dissolve the DNA.
11. Add 100 µl Buffer TE to dissolve DNA and store under appropriate conditions.

    Note: If DNA is dissolved slowly, keep it in 65°C water bath for 10-30 min to accelerate the dissolution.

Detection of DNA Concentration and Purity

The size of the obtained genomic DNA fragment is related to factors such as sample storage time and shearing force during operation. The concentration and purity of the purified DNA fragments can be detected by agarose gel electrophoresis and ultraviolet spectrophotometer.

DNA should have a significant absorption peak at OD_{260}, with OD_{260} value of 1 equivalent to about 50 µg/ml double stranded DNA and 40 µg/ml single stranded DNA.

The ratio of OD_{260}/OD_{280} should be 1.7-1.9. If deionized water is used for elution, the ratio will be low, because the pH value and the presence of ions will affect the light absorption value, but it does not mean the purity is low.