

Probe qPCR Detecting GMO Marker KK179 from Alfalfa

DESCRIPTION

Genetically modified (GM) alfalfa has been authorized for cultivation in several countries since 2005. However, cultivation in or export to some regions is not allowed. Therefore, event-specific detection is needed for GM alfalfa. The real-time qPCR method is sensitive and robust and is regarded as the gold standard for GMO analysis.

The Alfalfa KK179 qPCR Kit is designed for identifying GMO marker KK179 presence or absence in alfalfa crops, feed and seeds using real-time quantitative polymerase chain reaction (qPCR), primers, and labeled probes. The Kit includes maize KK179 Positive and Negative controls, and PCR internal controls, qPCR Super Mix, Alfalfa KK179 Prime-Probe Mix, in which the probe is labeled with Fam, and Hex is labeled for PCR internal control. This aids in the straightforward interpretation of the results.

PRINCIPLE

GMO marker identification is based on qPCR amplification of a specific region of the relevant target genome. The amplified product is detected using target-specific fluorescent probes that bind to the amplified product. As the PCR product accumulates, there is an increased fluorescence signal from the bound probes. Monitoring the fluorescence intensities during the PCR run allows the detection of the accumulating PCR product in real time.

KEY FEATURES

- ❖ Highly sensitivity and specificity for identification of KK179.
- ❖ High efficiency: the optimal systemic conditions for PCR amplifications.
- ❖ Streamlined protocol: Just add DNA Template, and water.
- ❖ No cross reactivity with others.

APPLICATIONS

Detect KK179 in alfalfa crop feed and seeds.

KIT CONTENTS

Name	100RXN
qPCP Super Mix	0.8 mL
Primer-probe Mix	0.3 mL
Positive Control DNA	60 µL
Negative Control DNA	60 µL

KK179 gene is labeled with FAM.
Alfalfa internal control is labeled with Hex.

STORAGE CONDITION

The kit is shipped on ice and stored at -20°C for long-term storage. Shelf life of 12 months after receipt.

PCR PROTOCOL

1. DNA extraction: The methods for DNA extraction can be used for any suitable preparation of DNA purification from food samples. We recommend that the Fast genomic DNA extraction method is used for this purpose (catalog: TBS6008).

2. Set up PCR reaction for each sample in 20 µL

Reaction Component	Volume (µL)
qPCP Super Mix	7.0
Primer-probe Mix	3.0
Nuclease-free Water	5.0
DNA sample	5.0
Final Volume	20 µL

Internal control should be included as below: Positive Control or Negative control (5 µL /reaction).

3. Suggested PCR conditions

Step	Amplification	PCR	
		CYCLE (40 cycles)	
	HOLD	Denature	Anneal/ Extend
Temperature	95 °C	95 °C	60 °C
Time	1 min	15 sec	60 sec

DATA ANALYSIS

Positive Reaction: Sample Ct < or = 30, and Positive, Negative and Blank controls are normal.

Negative Reaction: Sample Ct ≥ 37, and Positive, Negative and Blank controls are normal.

PCR internal control is positive in all samples, positive and negative controls. The positive response indicates a normal PCR amplification. Otherwise, the PCR reaction may be inhibited.

Repeat Reaction: If one of the control reactions is not normal, PCR reaction fails, and should be repeated.

RELATIVE PRODUCTS

- TBS6008: Fast Genomic DNA Extraction
- TBS6025: Microbial DNA Magnetic Extraction
- TBS43001: Maize GMO 98140 qPCR Detection
- TBS43003: Maize GMO DBT418 qPCR Detection
- TBS43004: Maize GMO LY038 qPCR Detection
- TBS43005: Maize GMO Mon863 qPCR Detection
- TBS43006: Maize GMO Mon87411 qPCR Detection
- TBS43007: Maize GMO DAS-59132 qPCR Detection
- TBS43008: Alfalfa J101 qPCR Detection
- TBS43009: Alfalfa J163 qPCR Detection
- TBS42025: 4-In-1 Aspergillus Species qPCR
- TBS42026: O157H7 E. Coli qPCR
- TBS42028: Salmonella qPCR
- TBS42029: Salmonella-STE C qPCR
- TBS42031: Listeria Monocytogen qPCR
- TBS42032: Listeria Species qPCR
- TBS42033: Bacillus Cereus qPCR

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