MBPCR055 Genetically Modified Organisms (GMO) Detection Kit

Description:

GMOs (Genetically Modified Organisms) are those organisms that have been modified by the application of recombinant DNA technology or genetic engineering, a technique used for altering a living organism's genetic material. With the rapid advances in biotechnology, a number of genetically modified (GM) crop or transgenic crops carrying novel traits have been developed and released for commercial agriculture production.

Intended Use

The kit is designed for *in vitro* diagnostics and provides qualitative detection. This diagnostic kit assures sensitive detection in environmental samples as well.

Product Description:

The GMO Detection Kit is designed for detection of specific sequences of **35S gene and *NOS* terminator gene** from various GMO food sources. The regulatory sequence of the cauliflower mosaic virus 35S (CaMV-35S) promoter and the *Agrobacterium tumefaciens* nopaline synthase gene (*NOS*) terminator are widely incorporated in genetically modified (GM) crops.

Conventional PCR testing can provide rapid, sensitive and specific detection of GMO kit. This kit also contains **internal control** and **Positive control.**

Internal control: This kit is provided with **rbcL gene**, DNA sequences obtained from chloroplast gene. This is a control sequence which is amplified in the same reaction tube along with the target sequence or in separate tubes. An internal control is often used to detect the failure of amplification in cases where the target sequence is not amplified.

Positive control: This is a control reaction using a known template. A positive control is usually used to check that the primers have been designed properly and the PCR conditions have been set up correctly.

Principle:

HiMedia's GMO Detection Kit is a qualitative conventional PCR kit which contains the amplification GMO samples using specific primers 35s and NOS terminator. The amplified target is detected by using agarose gel electrophoresis.

Polymerase Chain Reaction (PCR) is a very sensitive and specific method for amplification based detection of genes. The three steps of a successful PCR reaction include Denaturation, Annealing and Extension. The double-stranded DNA melts and forms single stranded DNA at high temperature

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Registered Office :
23, Vadhani Industrial Estate,LBS Marg,
Mumbai - 400 086, India.
Tel. : (022) 4017 9797 / 2500 1607
Fax : (022) 2500 2286

Commercial Office
A-516, Swastik Disha Business Park,
Via Vadhani Indl. Est., LBS Marg,
Mumbai - 400 086, India

Tel: 00-91-22-6147 1919
Fax: 6147 1920, 2500 5764
Email : info@himedialabs.com
Web : www.himedialabs.com

(Denaturation). Sequence-specific primers bind to the target sequence on single-stranded DNA at lower temperature (Annealing). Taq DNA Polymerase adds dNTPs onto the single stranded DNA at intermediate temperature (Extension). These 3 steps of PCR are usually repeated between 25 to 40 times in each PCR assay.

Gel electrophoresis is used to analyze the amplification of desired gene region for target pathogen based on separation of DNA fragments according to their size.

Features:

- Fast and simple
- Extremely sensitive and specific
- Guaranteed reproducible results

Unit Definition:

1U is defined as amount of enzyme that is required to catalyze the incorporation of 10 nmoles of dNTP into acid-insoluble material in 30 minutes at 74°C.

Storage and Shelf-life:

The provided kit has a shelf-life of 12 months when stored at -20°C. Repeated thawing and freezing of PCR reagents should be avoided, as this may reduce the sensitivity. If reagents are to be used multiple times, we recommend storing reagents as aliquots to avoid repeated freeze and thaw. Degradation of sample DNA specimens can also reduce sensitivity of the assay. HiMedia does not recommend using the kit after the expiry date stated on pack.

Kit Contents:

The provided PCR kit contains:

Components	Product Code	Reagents provided for 10R (reactions)	Reagents provided for 25R (reactions)	Reagents provided for 50R (reactions)
2X PCR TaqMixture	MBT061	550 µl	1300 µl	2.7ml
Primer Mix for 35s	DS0160	45 µl	110 µl	230 µl
Primer Mix for NOS gene	DS0161	45 µl	110 µl	230 µl
Primer Mix for Internal Control (rbcL gene)	DS0162	45 µl	110 µl	230 µl
Molecular Biology Grade Water for PCR	ML065	1 ml	2 ml	4ml
6X Gel Loading Buffer	ML015	100 µl	200 µl	400 µl
50 bp DNA Ladder	MBT084	40 µl	90 µl	180 µl
Internal Control DNA (rbcL gene)	DS0321	25 µl	55 µl	110 µl
Positive control (Bt Cotton)	DS0313	25 µl	55 µl	110 µl
Positive control (Non Bt Cotton)	DS0314	25 µl	55 µl	110 µl

Sample Collection and Preparation:
Various food source sample can be examined. For preparation of bacterial DNA, perform the nucleic acid purification using **HiPurA™ Plant Genomic DNA Miniprep Purification Kit (MB507)** as described in the protocol.

General Preparation Instructions:

- Before use, suitable amount of all PCR components should be completely thawed on ice (4°C).
- Perform the amplification reactions in a clean area.
- Use of aerosol barrier pipette tips is recommended to reduce contamination risks from extraneous DNA templates.
- Extract and store positive control material (if used) separately from all other reagents to avoid contamination and add it to the reaction mix in a separate area.
- Centrifuge the components briefly once thawed.

A) Protocol:

Preparation of PCR Reaction Mixture (UNIPLEX)

Perform PCR reactions for each DNA sample as per the following table:

Gene	**35S Promoter**	**NOS terminator + internal control (rbcL)**
Tube #	**#1**	**#2**
2X PCR TaqMixture **(MBT061)**	25µl	25µl
Primer Mix	2 µl	2µl NOS Primer
		2µl rbcLPrimer
Template DNA	1-2 µl	1-2 µl
Molecular Biology Grade Water for PCR **(ML065)**	Upto 50 µl	Upto 50 µl

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Centrifuge the tube briefly at 6000 rpm for about 10 seconds.

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Place the tubes in the PCR machine and set the recommended PCR program. (mentioned below)

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Interpret the data using Agarose gel electrophoresis

B. Recommended PCR program:

1. Initial denaturation : 94°C for 5 minutes

2. Cycling Parameters (No. of cycles: 30)
   Denaturation : 94°C for 30 seconds
   Annealing : 60°C for 30 seconds
   Extension : 72°C for 30 seconds

3. Final Extension : 72°C for 5 minutes

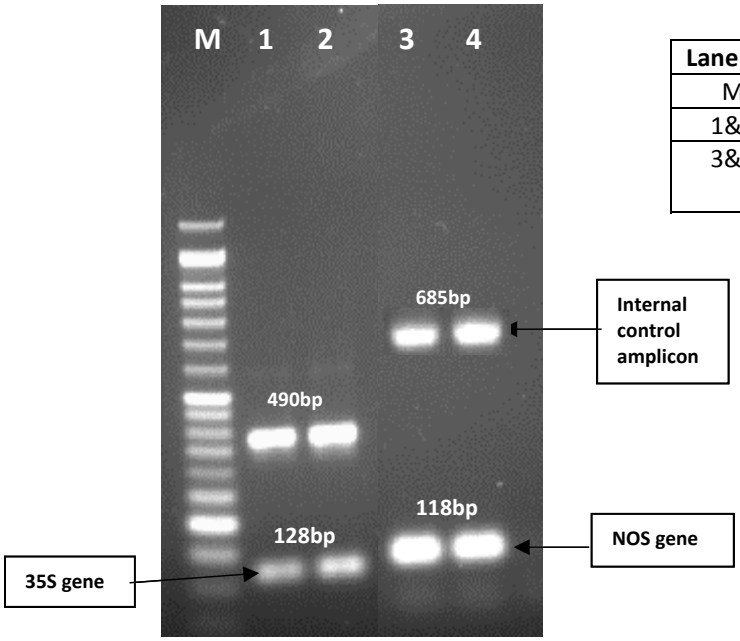
C. After amplification the products can be kept at 4°C overnight or frozen at –20°C for long-term storage.

D. GMO PCR Assay Results Interpretation

- For analysis of the PCR data, load 10 µl of amplicon on a 1.5% Agarose gel along with 1 µl of 6X Gel Loading Buffer (ML015)
- Load 3 µl of 50 bp DNA ladder (MBT084) in separate well

E. EtBr-staining to check results

- Incorporate EtBr in the agarose gel or stain the agarose gel with EtBr for 10-15 minutes
- Confirm the expected amplicon size comparing with 50 bp DNA marker



Lane no.	Samples
M	50 bp ladder
1&2	35S gene amplicon of GMO sample
3&4	NOS gene amplicon of GMO sample & rbcL gene amplicon as internal control

Image representing 35S gene (490 bp and 128 bp) using cotton GMO DNA, NOS gene (118 bp) and rbcL gene (685 bp)

Quality Control:

Each lot of HiMedia's GMO Detection Kit is assayed for contaminating endonuclease, exonuclease, and non-specific DNase activities. Functionally tested in DNA amplification.

Troubleshooting Guide:

Sr.No.	Problem	Cause	Solution
1.	No amplification	Degraded samples	1. Check the integrity of DNA using agarose gel electrophoresis.
			2. Use freshly prepared DNA to ensure the availability of intact template sequence for efficient amplification.
		Error in protocol setup	Verify that the correct reagent volumes, dilutions and storage conditions have been used.
2.	Variability between replicates	Error in reaction set-up	Prepare large volume reaction mix, vortex thoroughly and aliquot appropriately into reaction tubes.
		Air bubbles in reaction mix	Briefly centrifuge reaction samples/plate prior to running on a PCR machine.
		Pipetting error	Replicates can show increased variation due to poor laboratory techniques or imprecise pipettes.
3.	Amplification in negative control	Reagents contaminated	1. Replace all critical solutions 2. Repeat the analysis of all tests with fresh aliquots of critical reagents.

Safety Information

The GMO Detection Kit is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

Product Use Limitation & Warranty

HiMedia guarantees the performance of product in the manner described in the product literature. GMO Detection Kit is designed and sold for research and *in vitro* purposes only. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of HiMedia products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Technical Assistance

At HiMedia we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance mail at mb@himedialabs.com

Consult instructions for use

Do not use if package is damaged

HiMedia Laboratories Pvt. Limited,
Reg. Off: 23 Vadhani Industrial Estate,
LBS Marg, Mumbai - 400086,
India Works: B/4-6, M.I.D.C., Dindori,
Nashik, India (or respective plant address)
Customer Care No: 022-6116 9797
www.himedialabs.com

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