MBPCR055 Hi-PCR® GMO (Genetically Modified Organism) Semi-Q PCR Kit

Description:

Genetically Modified Organisms (GMOs) are organisms whose genetic material is altered through recombinant DNA technology to introduce specific traits, such as pest resistance or enhanced nutritional content. Advancements in biotechnology have driven the widespread adoption of GM crops, including soybeans, corn, and cotton, which now occupy 190 million hectares globally, particularly in the United States, Brazil, Argentina, India, and China. These crops, engineered with novel traits, have become integral to global agriculture.

With the rapid growth in global trade of GM ingredients and stricter regulatory requirements for GMO labeling, accurate detection of GMOs in food and feed sources has become essential to ensure compliance and safety.

Intended Use

The Hi-PCR® GMO Semi-Q PCR Kit is intended for the qualitative detection of genetically modified (GMO) DNA in food and feed samples.

Product Description:

The Hi-PCR® GMO (Genetically Modified Organism) Semi-Q PCR Kit is designed for detection of specific sequences of **35S gene (195 bp) and *NOS* terminator gene (118 bp)** 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. Hence, this kit can screen >80% of GM food sources that are generally constructed with these gene targets.

The kit also includes internal and positive controls to ensure the reliability and accuracy of the test results.
Internal control: The internal control provided in this kit is the **rbcL gene (654 bp)**, a chloroplast gene sequence. This control is co-amplified with the target GMO sequences in the same PCR reaction, ensuring that the PCR process is functioning correctly. An internal control is often used to detect the failure of amplification in cases where the target sequence is not amplified.

Positive control: The positive control is a known template that contains the target GMO sequences. This control verifies that the PCR conditions have been set up correctly.

No Template Control: A no template control is run to ensure that the reagents, equipment, and environment used in the assay are not contaminated with target DNA. In this reaction, PCR grade water is used as the template.

Principle:
The Hi-PCR® GMO Semi-Q PCR Kit is designed to detect the presence of specific GMO markers, namely the 35S promoter gene from the cauliflower mosaic virus (CaMV) and the NOS terminator gene from Agrobacterium tumefaciens, which are commonly used in genetically modified crops. The Hi-PCR® GMO Semi-Q PCR Kit operates on the principle of conventional PCR, a highly sensitive

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and specific method for gene amplification and detection. The amplification of DNA is achieved using sequence-specific primers that bind to the 35S promoter and NOS terminator genes.
The PCR process involves three key steps:
1. Denaturation: The double-stranded DNA is heated to a high temperature, causing the DNA to melt into two single strands.
2. Annealing: The reaction temperature is lowered, allowing the sequence-specific primers to bind to the target DNA sequences.
3. Extension: Taq DNA polymerase extends the primers by adding nucleotides to the single-stranded DNA, resulting in the amplification of the target sequence.
These steps are repeated multiple times (typically 25 to 40 cycles) to exponentially amplify the target sequences. Following amplification, gel electrophoresis is used to analyze the PCR products. This technique separates DNA fragments based on size, allowing for the visualization and confirmation of the target gene's presence.

Features:

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

Storage and Shelf-life:

The provided kit has a shelf-life of 12 months when stored between -10°C to -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 reactions * (µL)	
		25R	50R
GMO Semi-q Master Mix	DS1921	216	432
Primer Mix for 35s	DS0160	54	108
Primer Mix for NOS gene	DS0161	54	108
Primer Mix for Internal Control (rbcL gene)	DS0162	54	108
Molecular Biology Grade Water for PCR	ML065	702	1404
6X Gel Loading Buffer	ML015	108	216
50 bp DNA Ladder	MBT084	15	30
Positive control (GMO)	DS0313	10	20
Positive control (non-GMO)	DS0314	10	20

*For a 20 µL reaction

Materials needed but not provided:

- PCR tubes (Product code PW1255) or PCR Strips (Product code: PR17) or PCR Plates (Product code: PR2 / PR3 / PR19)
- Thermal Cycler (Product Code: LA948/LA949/LA950/LA1006/LA1015/LA1059/LA1060/LA1066)
- Barrier Micropipette Tips (Product Code: LA749 / LA749A / LA751 / LA751A / LA750 / LA750A / LA859 / LA859A)
- Micropipettes

Sample Collection and Preparation:

Various food source sample can be examined. Kits that have been validated for extraction of GMO and non-GMO DNA include HiPurA® Plant Genomic DNA Miniprep Purification Kit (MB507), HiPurA Pre-filled catridges for plant DNA purification (MB507PC16) or HiPurA® Pre-filled Plates for Plant DNA Purification (MB507MPF16/ MB507MPF-32/ MB507MPF-96).

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:

<u>Preparation of PCR Reaction Mixture</u>

Two separate tubes have to be run for a single sample/ Positive control/ NTC.
Perform PCR reactions for each DNA sample as per the following table:

	Tube 1 (35S Promoter)	Tube 2 (NOS terminator + rbcl (internal control))
GMO Semi-q Master Mix (DS1921)	4 µl	4 µl
Primer Mix for 35s (DS0160)	2 µl	-
Primer Mix for NOS gene (DS0161)	-	2 µl
Primer Mix for Internal Control (rbcL gene) (DS1062)	-	2µl
Molecular Biology Grade Water for PCR (ML065)	13 µl	11 µl
Template DNA/Positive control/NTC*	1 µl*	1 µl*
Total volume	20 µl	20 µl

*** For NTC tube use Molecular Biology Grade Water for PCR (provided) in place of Template DNA. Use Template DNA concentration of 5ng/µl for consistent results**

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 : 58°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 2.0 % 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.

F. Result Interpretation:

Tube-1	**Tube-2**		**Result Interpretation**
35S amplicon	**NOS amplicon**	**rbcL amplicon**	
195 bp, 490 bp*	118 bp	654 bp	GMO Positive
-	-	654 bp	Non-GMO
-	-	-	PCR inhibition or reagent failure or DNA extraction issue

*- **An additional band of 490 bp may be observed in certain GMO samples due to use of hybrid plant source with different 35S promoter sequence**

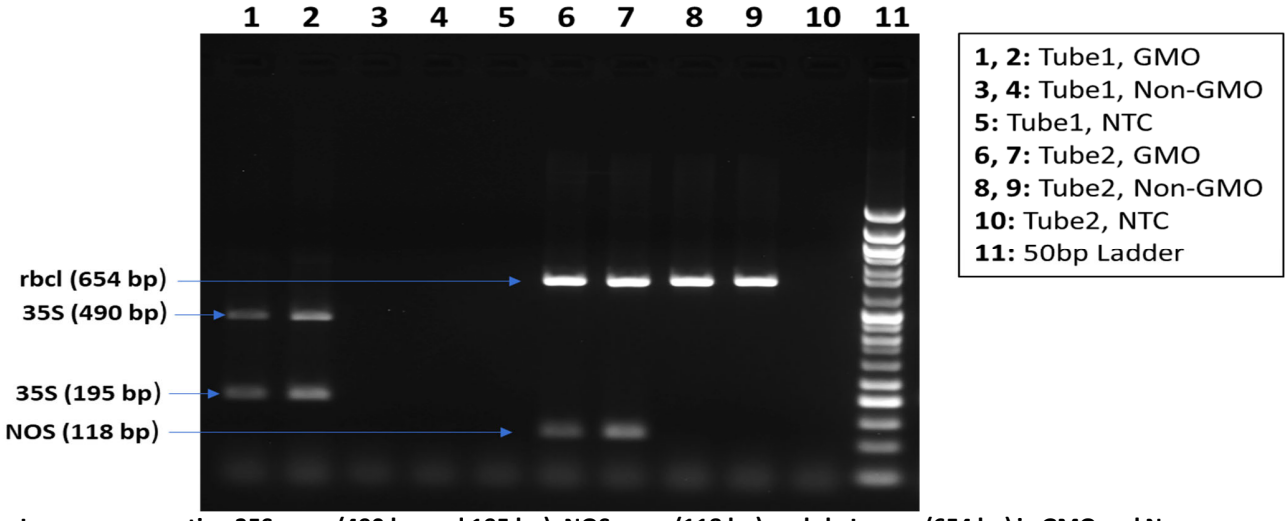

Image representing 35S gene (490 bp and 195 bp), NOS gene (118 bp) and rbcL gene (654 bp) in GMO and Non-GMO samples.

Quality Control:

Each lot of HiMedia's Hi-PCR® GMO (Genetically Modifed Organism) Semi-Q PCR Kit is 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 Hi-PCR® GMO (Genetically Modifed Organism) Semi-Q PCR 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. Hi- PCR® GMO (Genetically Modifed Organism) Semi-Q PCR Kit is designed and sold for research and *in vitro* purposes only. This kit is designed for detection of GM food sources built with 35S promoter from Cauliflower mosaic virus (CaMV) and/or the NOS terminator derived from *Agrobacterium tumefaciens*. Hence GMOs that are constructed with other gene targets can be missed.
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 mb@himedialabs.com.

Symbols

	Manufacturer		Do not use if package is damaged
	Batch code		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		Catalogue number (commercial product name", commercial product code")

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