

MBPCR063 Hi-PCR® GMO (Genetically Modified Organism) SYBr 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 have become essential to ensure compliance and safety.

Intended Use:

Recommended for the qualitative detection of GMO in food and feed samples.

Product Description:

HiMedia's Hi-PCR® GMO (Genetically Modified Organism) SYBr PCR 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. Hence, this kit can screen >80% of GM food sources. Real-time PCR testing can provide rapid, sensitive and specific detection of GMO. This kit also contains an internal control as well as a positive control.

Principle:

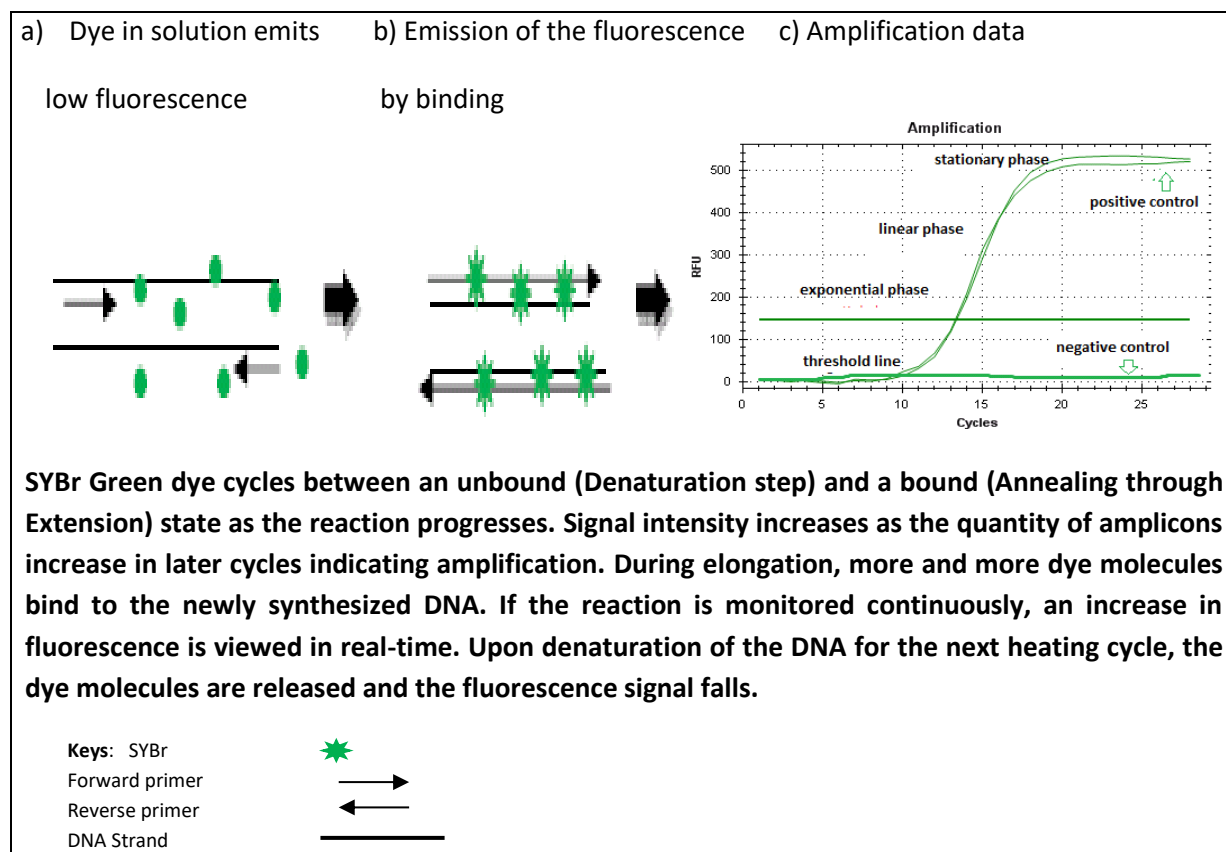
Real-time Polymerase Chain Reaction, also called quantitative Polymerase Chain Reaction (qPCR) or kinetic Polymerase Chain Reaction, is a laboratory technique based on the principle of Polymerase Chain Reaction. This technique is used to amplify and simultaneously quantitate a targeted DNA sequence. Real-time PCR systems based on SYBr Green assays have increasingly been used for accurate, reliable detection and quantitation of various food-borne pathogens.

Internal control: This kit includes primers that amplify a specific portion of plant chloroplast gene, acting as an internal positive control. This is a control sequence which is amplified in separate tube. 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 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.

Diagrammatic representation of preferential binding of SYBr Green Dye to specific DNA fragments in real-time PCR.



SYBr Green dye cycles between an unbound (Denaturation step) and a bound (Annealing through Extension) state as the reaction progresses. Signal intensity increases as the quantity of amplicons increase in later cycles indicating amplification. During elongation, more and more dye molecules bind to the newly synthesized DNA. If the reaction is monitored continuously, an increase in fluorescence is viewed in real-time. Upon denaturation of the DNA for the next heating cycle, the dye molecules are released and the fluorescence signal falls.

Features:

- Fast and simple
- Sensitive and specific results
- Guaranteed reproducible results

Sample Source: Plant and food samples

Storage:

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 SYBr Master Mix	DS1920	810	1620
Primer Mix for 35S	DS1503	27	54
Primer Mix (NOS gene)	DS2160	27	54
Primer Mix for Internal Control (rbcl gene)	DS0321	27	54
Molecular Biology Grade Water for PCR	ML065	750	1500
Positive control (GMO)	DS0313	15	30
Positive control (Non-GMO)	DS0314	15	30

*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) & Sealing film (PR18)
- Insta Q Real Time PCR System (Product Code: LA1012/LA1073/LA1023/LA1024/LA1074)
- Barrier Micropipette Tips (Product Code: LA749 / LA749A / LA751 / LA751A / LA750 / LA750A / LA859 / LA859A)
- Micropipettes

Specimen collection and Handling:

Follow appropriate techniques for handling specimens; after use, contaminated materials must be sterilized by autoclaving before discarding. Standard precautions as per established guidelines should be followed while handling clinical specimens and items contaminated with blood and other body fluids. Safety guidelines may be referred to in individual safety data sheets.

Sample Collection and Preparation:

Various food and feed samples 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 cartridges for plant DNA purification (MB507PC16) or HiPurA® Pre-filled Plates for Plant DNA Purification (MB507MPF16/ MB507MPF-32/ MB507MPF-96).

General Preparation Instructions:

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

A. Protocol for PCR Master Mix Preparation

Three 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:

Component	Product code	35S Promoter	NOS Terminator	Internal Control
		Tube 1	Tube 2	Tube 3
GMO SYBr Master Mix	DS1920	10.0 µL	10.0 µL	10.0 µL
Primer Mix for 35S	DS1503	1.0 µL	-	-
Primer Mix (NOS gene)	DS2160	-	1.0 µL	-
Primer Mix for Internal Control (rbcL gene)	DS0321	-	-	1.0 µL
Molecular Biology Grade Water for PCR	ML065	8.0 µL	8.0 µL	8.0 µL
Template DNA/Positive control/NTC*	-	1.0 µL	1.0 µL	1.0 µL
	Total	20.0 µL	20.0 µL	20.0 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.



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



Interpret the data from the amplification plot (observe the Ct values).

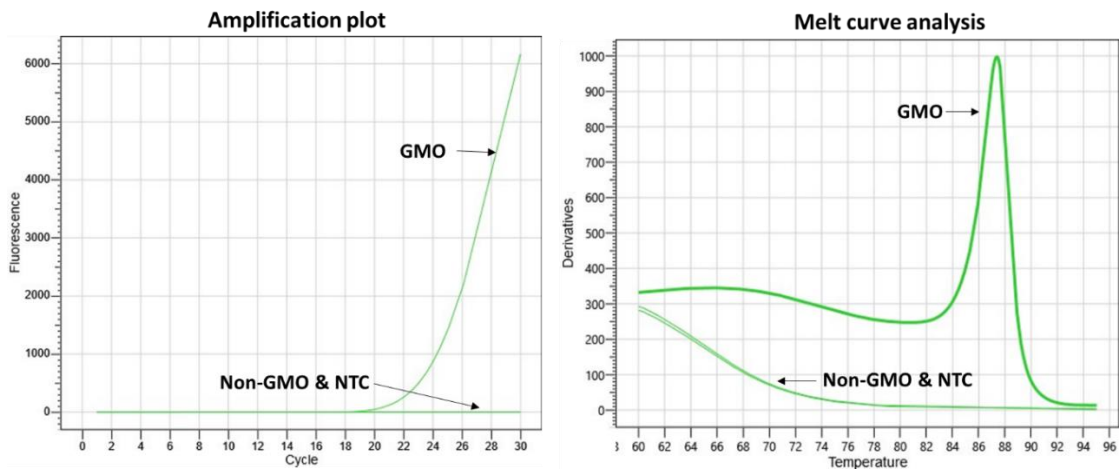
B. Recommended PCR program

- | | | | |
|---|---|---------------------|---------------------|
| 1. Initial denaturation | : | 94°C for 5 minutes | No. of cycles: 01 |
| 2. Denaturation | : | 94°C for 30 seconds | } No. of cycles: 30 |
| 3. Annealing (Plate Read) | : | 58°C for 30 seconds | |
| 4. Extension | : | 72°C for 30 seconds | |
| 5. Melt Curve Analysis as per HiMedia's Insta Q96 Real-Time PCR Machine | | | |
| 95°C | : | 15 seconds | |
| 60°C | : | 1 minute | |
| 95°C | : | 15 seconds | |
| Increment | : | 0.5°C | |
| On Hold | : | 10 seconds | |
| 6. Hold | : | 4°C for ∞ | |

NOTE: The user can also set up a melt curve program as per their existing PCR instrument.

C. Amplification Data

35S Target:



Sr. No.	Sample	Avg. C _t value	Avg. T _m (°C)
1	GMO DNA	25.5	87.37
2	Non-GMO	-	-
3	NTC	-	-

Figure 1: Data representing real-time amplification of 35S gene with GMO DNA and Non-GMO DNA with C_t and T_m values (provided in table) run on Insta Q96® Plus Real Time PCR Machine.

NOS Target:

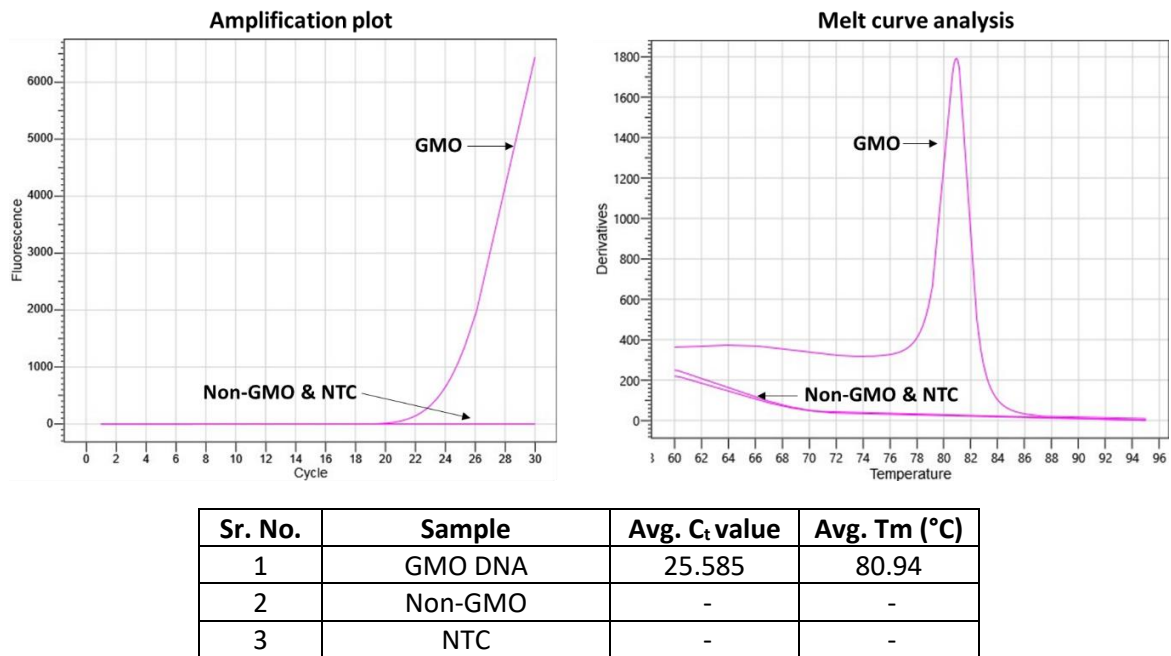


Figure 2: Data representing real-time amplification of NOS gene with GMO DNA and Non-GMO DNA with C_t and T_m values (provided in table) run on Insta Q96® Plus Real Time PCR Machine.

IC Target (rbcl):

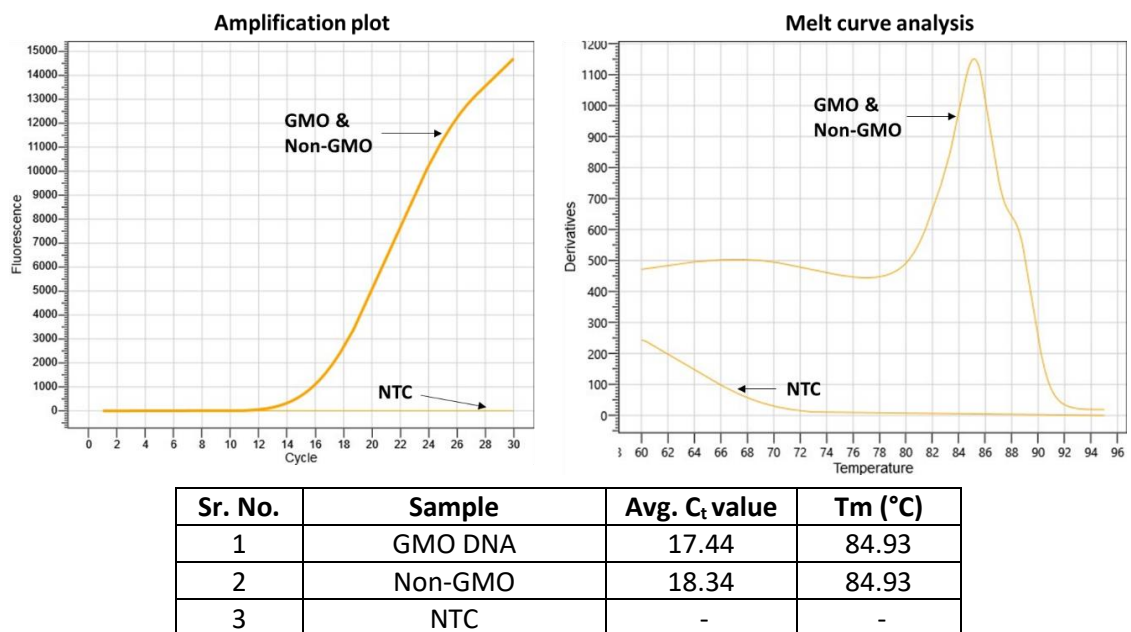


Figure 3: Data representing real-time amplification of IC with GMO DNA and Non-GMO DNA with C_t and T_m values (provided in table) run on Insta Q96® Plus Real Time PCR Machine.

Data Interpretation:

Gene	Melting Temperature (T _m)*	Result Interpretation
35S	85.0°C – 88.5°C	GMO Positive
NOS	79.5°C – 82.0°C	GMO Positive
rbcl	83.5°C – 86.0°C	Positive for IC gene

* T_m values can slightly vary for different sample types. If the T_m values show significant variation from those mentioned in the above table, then the sample is considered to be negative for respective genes.

Precautions:

Read the procedure carefully before starting the experiment. Wear protective gloves/protective clothing/eye protection/face protection. Follow good laboratory practices while handling samples. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred to in safety data sheets of the product.

Quality Control:

Each lot of HiMedia’s Hi-PCR® GMO (Genetically Modified Organism) SYBr PCR Kit is functionally tested in DNA amplification.

Performance and Evaluation:

Each lot of HiMedia’s Hi-PCR® GMO (Genetically Modified Organism) SYBr PCR Kit is tested against pre-determined specifications to ensure consistent product quality.

Analytical Sensitivity - Limit of Detection (LoD)

The Limit of Detection (LoD) is defined as the concentration (copies per µl of the eluate) of target molecule that can be detected at 95% or greater probability. The LoD assay for the Hi-PCR® GMO (Genetically Modified Organism) SYBr PCR Kit was performed using Synthetic DNA by preliminary testing a 10-fold dilution series in triplicates per concentration followed by confirmation with 20 replicates of the concentration determined to be the LoD. The detectable Limit of Detection (LoD) of 35S and NOS targets was determined to be **250 copies/µL and 100 copies/µL**, respectively.

Troubleshooting Guide:

Sr. No.	Problem	Cause	Solution
1.	No amplification	Degraded samples	Check the integrity of DNA using agarose gel electrophoresis. 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 master mix, vortex thoroughly and aliquot into reaction tubes.
		Air bubbles in reaction mix	Briefly centrifuge reaction samples/plate prior to running on a real-time PCR instrument.
		Pipetting error	C _t values of replicates can show increased variation due to poor laboratory technique 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.
4.	No signal with Positive controls	Incorrect programming of the temperature profile of the thermal cycler	Compare the temperature profile to the manual.

Safety Information;

The Hi-PCR® GMO (Genetically Modified Organism) SYBr PCR Kit is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

Disposal:

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures for the disposal of biological material. Material that are potentially contaminated and/or infectious must be decontaminated and disposed of in accordance with current laboratory techniques.




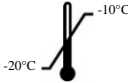




Product Use Limitation & Warranty:

HiMedia guarantees the performance of product in the manner described in the product literature. Hi-PCR® GMO (Genetically Modified Organism) SYBr 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 at 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

Identification No.: PIMBPCR063

Rev.No.:13

Date of Issue: 2025-07

Disclaimer :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory, diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.

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