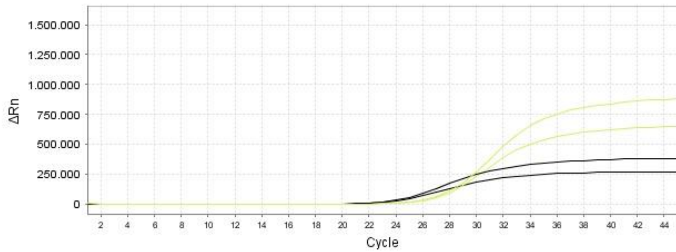
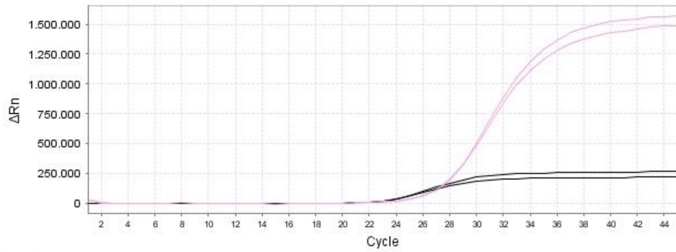
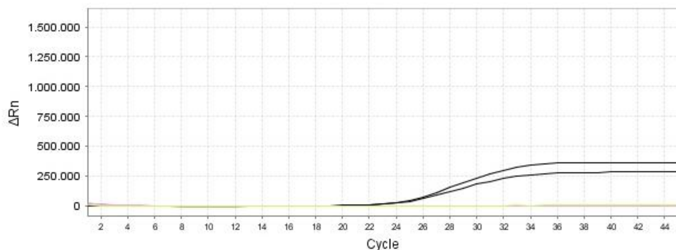
Positive Control –
P-35S



T-NOS



Negative Control –



12. SPECIFICITY/INCLUSIVITY

a) 100 % Exclusivity, determined using DNA from non-target GMOs and other vegetables suitable to occur in the same food products; 18 non-target species for P-35S and 21 non-target species for T-NOS.
b) 100 % Inclusivity, determined in 9 positive samples.

13. SENSITIVITY

The limit of detection (LoD) for REAL TIME DETECTION KIT P-35S and T-NOS is 25 pg (P-35S) and 50 pg (T-NOS) of target DNA. The method's detection limit can detect 0,05% (P-35S) and 0,1% (T-NOS) of the target GMO in 50 ng of total DNA. No false negatives were obtained, either in the defined PCR conditions or in the deviations used for robustness, increasing the stringency.

BPMR – Production and Development, Lda. Amadora INOVA
Rua Henrique de Paiva Couceiro, 10
2700 – 453 Falagueira – Venda Nova Amadora, PORTUGAL
Tel: +351 211 451 410
E-mail: sales.support@biopremier.com
Website: http://www.biopremier.com

BPMR is certified ISO 9001:2015

MKT-0022 Last revision: September 2023

BIOPREMIER

REAL TIME DETECTION KIT
P-35S and T-NOS

Ref: BIOPFS-0016

1. PRODUCT DESCRIPTION

GMOs detection kits provide a simple, reliable, and rapid procedure for detecting the presence of a specific target. The assay is based on 5' nuclease real time PCR reactions to amplify a unique genomic sequence in the target microorganism.

2. TECHNOLOGY DESCRIPTION

PCR is a method used to amplify a specific DNA sequence in a reaction containing among other components, a thermostable DNA polymerase, nucleotides, and primers complementary to the target sequence. The DNA molecule denatures when this solution is heated, separating into two strands. As the solution cools, the primers anneal to the target sequences in the separated DNA strands and the DNA polymerase synthetizes a new strand by extending the primers with nucleotides, creating a copy of the DNA sequence (amplicons). When repeated, this cycle of denaturing, annealing, and extending exponentially increases the number of target amplicons. In Real Time PCR, the signal is measured in each cycle, using in most cases specific fluorescent probes. Fluorescence is measured by a detector and the associated software plots the fluorescence intensity versus the number of cycles, allowing the determination of the presence or absence of the target organism.

3. SCOPE

Genetically modified food and feed products have become a reality. Since 1994, the date of their initial commercialization, about 150 genetically modified plants have received approval for use as food or feed. Their use is highly regulated with the implementation of compliance measures. For the enforcement of this legislation is essential an efficient detection of genetically modified organisms (GMOs) in food and feed products. Considering their large diversity (different GM elements in various species), the application of a generic screening for the presence of GM material is most often the first step in GMO analysis. Such qualitative screening methods provide a presence/absence response and help reduce the number of subsequent identification analyses. The promoter 35S obtained from cauliflower mosaic virus (CaMV) and the terminator NOS from *Agrobacterium tumefaciens* are the most frequent elements present in transgenic material in food. Real-time PCR methods targeting these elements have shown to be a reliable strategy to perform this screening.

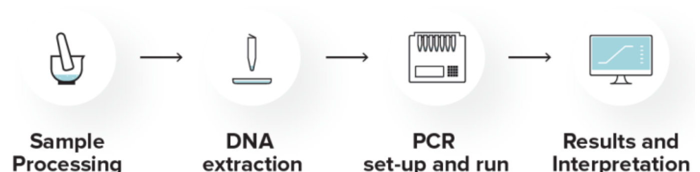
4. INTERNAL CONTROL (IC)

GMOs detection kits include an Internal Control (IC) in the master mix. This control is amplified at the same time as the target DNA sequence, but using a different set of primers and a probe labeled with a different fluorophore. The inclusion of the IC in each reaction allows the evaluation of PCR inhibitors in negative results.

5. APPLICATION

REAL TIME DETECTION KIT P-35S and T-NOS is intended for the rapid detection of P-35S and T-NOS elements in food and feedstuff products, after DNA extraction. It can also be used with other samples in which GMO detection is needed. The kit was validated in the instruments ABI PRISM® 7500 Fast and ThermoScientific® PikoReal. The kit is compatible with all thermocyclers working in **FAM** and **ROX** channels. The detection kit must not be used for diagnostic procedures.
For Food use only.

The procedure includes the following main steps:



6. CONTENTS AND STORAGE

The kit contains reagents for 100 assays for each target

CONTENTS	UNITS	COMPOSITION
Master Mix (blue cap)	4 tubes (4 x 840 µl)	Buffer, dNTPs, DNA polymerase
Assay Mix – P-35S (brown cap)	1 tube (1 x 210 µl)	Primers and Probes
Assay Mix – T-NOS (brown cap)	1 tube (1 x 210 µl)	Primers and Probes
Negative Control (clear cap)	1 tube (1 x 140 µl)	Nuclease-free water
Positive Control (red cap)	1 tube (1 x 140 µl)	Target DNA

Store all contents at -20ºC and protect them from light as excessive exposure to light may affect the fluorescent probes. Minimize freeze-thaw cycles. Reagents stored as recommended may be used until the expiration date indicated in the tube.

7. MATERIAL REQUIRED AND NOT SUPPLIED

- Microcentrifuge
- Laminar Air Flow Cabinets/PCR Cabinets
- Disposable powder-free gloves
- Micropipettes and nuclease-free filter tips
- Real-time PCR instrument
- Tubes/Strips/Multiwell plates and accessories specific for each Instrument
- DNA extraction kit (example: BIOPEXT-0609).

8. PRECAUTIONS AND RECOMMENDATIONS

Molecular Biology procedures, such as DNA extractions and PCR amplification, require qualified staff to prevent the risk of erroneous results, especially due to sample contamination or degradation of the nucleic acids contained in the samples. It is strongly recommended to have dedicated areas, materials, and equipment for the DNA extraction, preparation of the PCR and post-PCR procedures. Workflow in the laboratory must proceed in a unidirectional manner, beginning in the Extraction Area and moving to the Amplification and Detection Area.

The user should always pay attention to the following:

- Read all the instructions provided before running the assay.
- Do not mix reagents from different batches.
- Wear proper PPE, including disposable gloves and laboratory coats.
- Store and extract positive material separately from all other reagents.

9. QUALITY CONTROL

In accordance with BPMR's ISO 9001, each lot of the kit is tested against predetermined specifications to ensure consistent product quality.

10. PROCEDURE

10.1 Sample preparation

Depending on the specific characteristics of the sample, procedures like homogenization and grinding may be necessary before DNA extraction.

10.2 DNA extraction

Use a kit or protocol suitable for DNA extraction from food products, such as BIOPREMIER DNA Extraction Kit from Food (ref: BIOPEXT-0609) or similiar. Follow the manufacturer's or authors instructions.

10.3 PCR preparation

A – PCR mix

Always wear gloves for all PCR procedures.

1. Thaw the kit solutions. Mix thoroughly and centrifuge briefly
2. Prepare the reactions, as described below:

CONTENTS	Nº OF SAMPLES	
qPCR reaction	**1**	**10 (10 + 1)**
Master Mix	16 µl	176 µl
Assay Mix – P-35S or Assay Mix – T-NOS	2 µl	22 µl
Total Volume	**18 µl**	**198 µl**

Note: Prepare the PCR reaction for each sample, or in the alternative, prepare a Master Mix for the total number of reactions plus 10% to cover pipetting losses (e.g. for 10 samples, prepare a volume for 11). In this case, prepare the Mix in a 1,5mL sterile, nuclease-free tube. Include 2 PCR reactions for the Positive and Negative controls.

3. Mix the prepared Mix by inverting the tube and centrifuge briefly
4. Dispense 18 µL aliquots of prepared Mix into the plate wells or PCR tube
5. For the negative control, pipette 2 µL of Negative Control tube (Clear Cap); Pipette 2 µL of DNA sample per well; and for the positive control, pipette 2 µL of Positive Control tube (Red Cap). Each PCR tube / well should have a final PCR volume of 20 µL
6. Centrifuge briefly the plate wells or PCR tubes
7. Place the reactions into the Real Time PCR instrument.

B – Program set up

Prepare the Real-Time PCR instrument according to the following temperature/time program:

PHASE	STEP	TEMPERATURE	TIME	ACQUISITION
Holding Stage	Step 1	50ºC	2 min.	No
	Step 2	95ºC	5 min.	No
Amplification - 45 cycles	Step 1	95ºC	30 sec.	No
	Step 2	60ºC	30 sec.	Yes
	Step 3	72ºC	30 sec.	No

TARGET	CHANNELS
Detection of P-35S or T-NOS	FAM
Detection of Internal Control (IC)	ROX

11. DATA ANALYSIS

For analysis of PCR results, select fluorescence display options. Samples with positive Ct-values are considered positive.

Important: Please, also check amplification curves, not only Ct values. Samples should be inspected both in logarithmic and linear scale view and compared with the negative control. Adjust the Threshold, if necessary. Assessment of sample results should be performed after the positive and negative controls have been examined and determined to be valid. If the results of controls are not valid, the sample results cannot be interpreted.

Interpretation of PCR data:

a) Controls

To validate the assay, the controls must have the following results:

	P-35S or T-NOS DETECTION FAM	IC DETECTION ROX
Negative Control	Negative	Positive
Positive Control	Positive	Positive

Note that if the controls do not match these results, the experiment must be repeated.

b) Samples

Interpretation of sample results is summarized in the following table:

P-35S or T-NOS DETECTION FAM	IC DETECTION ROX	INTERPRETATION
Positive	Positive/Negative**	DNA detected, sample Positive for P-35S or T-NOS
Negative	Positive	No DNA detected, sample Negative for P-35S or T-NOS
Negative	Negative	Invalid Result*

*When both P-35S or T-NOS and IC detection are Negative, this means the presence of PCR inhibitors in the sample. Dilute the DNA sample (10-fold dilution) or perform another DNA extraction and repeat qPCR.

** High DNA concentration of the target in the sample can lead to a reduced or absent fluorescence signal of the IC.