Instructions for use

InviScreen® GMO Detection Kit (*35S/NOS/FMV/pat/bar*)

INVITEK diagnostics

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REF 6014004203 | Σ 6 x 50 tests | -25 °C / -15 °C | RUO

1. Intended use

InviScreen® GMO Detection Kit (35S/NOS/FMV/pat/bar) is designed for the qualitative detection of genetically modified organisms (GMOs) in food and feed samples. This kit is intended for use by trained laboratory professionals. It detects the most commonly used regulatory elements in genetically modified plants, namely the 35S promoter from cauliflower mosaic virus (pCamV35S), the NOS terminator from *Agrobacterium tumefaciens* (tNOS), the 34S promoter from figwort mosaic virus (pFMV), and the phosphinothricin N-acetyl transferase genes pat and bar, as well as an ubiquitous plant target. The PCR kit provides a reliable and sensitive method for the detection of GMOs in various food matrices, including but not limited to crops, processed food products, and animal feed. This kit is intended for screening purposes and can assist with meeting the labeling requirements for GMOs in the European Union (EU directive 1829/2003). However, it is important to note that additional regulatory compliance measures, such as quantification and validation of GMO content, may be necessary to meet specific EU labeling regulations.

2. Product description

InviScreen® GMO Detection Kit (35S/NOS/FMV/pat/bar) provides a real-time PCR method for the screening of GMO DNA sequences in food and feed. The kit combines the multiplexed detection of pCaMV35S/tNOS, pat/bar and pFMV to perform a thorough and complete qualitative detection of the most frequently used transgenic regulatory regions. The kit includes a plant-specific primer set to control the entire analytical process from nucleic acid extraction to DNA amplification. The real-time PCR method can be performed in commonly available PCR instruments with FAM and VIC/HEX fluorescence channels. Under optimal conditions, the kit provides highly sensitive detection of at least 0.1% relative GMO content.

3. Kit contents

REF.	COMPONENT	FUNCTION	CAP COLOR	QUANTITY
D31.01	Primer/Probe Mix – pCaMV35S/tNOS [1]	Targeted detection		1 tube, 500 µL
D31.02	Primer/Probe Mix – pat/bar [1]	Targeted detection		1 tube, 500 µL
D31.03	Primer/Probe Mix – pFMV [1]	Targeted detection		1 tube, 500 µL
D31.04	Primer/Probe Mix – Plant [1]	Targeted detection		1 tube, 500 µL
D31.05	qPCR Master Mix [1]	Amplification		2 tubes, 1000 µL
D31.06	Negative Control	Negative Control		1 tube, 200 µL
D31.07	Positive Control	Positive Control		1 tube, 200 µL

[1] Reagents are supplied with a 5% of extra volume

4. Storage

Reagents should be stored sealed at -20 ± 5°C and may be used until the expiration date shown on the package label. Expiry date refers to the product under rightful handling and storage conditions. It is not recommended the use of the kit after the expiry date stated on the box. Avoid unnecessary repeated freeze/thawing cycles. Protect reagents from light exposure to prevent degradation.

5. Equipment and materials required (Not provided)

- Food processor [2]
- DNA extraction kit
- Real-Time PCR instrument [3]
- Spectrophotometer/Fluorometer [4]
- Plates and/or tubes for qPCR
- 1.5 mL microcentrifuge tubes
- PCR cabinet [5]
- Micropipettes (10, 200 and 1000 µL) and filter tips
- Vortex and microcentrifuge

[2] It is recommended to carefully homogenize samples that are contain more than one ingredient in its composition and are not originally in a powdered or granulated form.
[3] The assay was validated on a Bio-Rad CFX96.
[4] It is recommended to quantify and access the purity of DNA.
[5] To minimize the risk of contamination with foreign DNA, we recommend that the PCR reaction set-up is performed in a controlled environment recommend that the PCR reaction set-up is performed in a controlled environment.

6. Suitable test sample material

All kind of food and feed samples suitable for Real-Time PCR amplification can be used, as long as the quality parameters for purity, concentration and integrity are fulfilled. It is recommended to carefully homogenize samples that contain more than one ingredient in its composition. In either case, it is necessary to adjust the quantity of sample in accordance to the manufacturer instructions of the total genomic DNA extraction kit in use. The use of InviSorb® Spin Plant Mini Kit for DNA extraction is highly recommended.

7. Test Procedure

a. PCR Reaction Preparation

Allow all reagents to thaw at room temperature, vortex and spin briefly to avoid drops on the vial cap. For each DNA sample prepare four reaction mixes[6] according to the table below and considering the table in point 3:

REAGENT	VOLUME
qPCR Master Mix	10 µL
Primer/Probe Mix	10 µL
Total Volume	**20 µL**

[6] For each sample, four PCR reactions (one for each set of targets) should be prepared.

1. Homogenize the reaction mixtures and pipette 20 µL into individual wells according to the predicted PCR plate set-up.
2. Add 5 µL of DNA template to each well. The ideal concentration of DNA is 30 ng/µL. Total DNA added to the PCR reaction should never exceed 150 ng.

At least one positive control reaction and one negative control reaction must be included in the PCR run, replacing the sample in these wells with 5 µL of Positive Control and 5 µL of Negative Control, respectively.

It is recommended to prepare the reaction mixture carefully in a controlled environment, preferably in a nucleic acid-free zone. The addition of the positive control and sample DNA should preferably be carried out in a separate room.

b. Amplification Protocol

The amplification conditions are as follows:

	STEPS	TEMPERATURE	TIME	CYCLES
1	Enzymatic Activation	95 °C	10 min	1
2	Denaturation	95 °C	30 seg	45
3	Hybridization/extension plate reading *	59 °C	1 min	

* Fluorescence data must be obtained during this step through FAM (tNOS, bar, pFMV, Plant) and HEX (pCaMV35S, pat, IAC) channels

c. Results Interpretation

The results should be interpreted in accordance with the analysis software recommended by the Real-Time PCR instrument manufacturer. The software monitors DNA amplification through the detection of fluorescence emitted by each probe, attributing a Ct value for each reporter dye found in each individual sample. Target DNA amplification is monitored in FAM and HEX/VIC channels. After setting the threshold baseline, the analysis outcome should be interpreted according to the scenarios referred bellow.

A result is considered positive when Ct ≤ 40.

RESULT	Target						
	p35S [9]	tNOS	pFMV	pat	bar	Plant [10]	IAC
Positive all targets	+	+	+	+	+	+	+/-
Positive p35S+pFMV+pat+bar	+	-	+	+	+	+	+/-
Positive p35S+tNOS+pat+bar	+	+	-	+	+	+	+/-
Positive p35S+tNOS+pFMV+bar	+	+	+	-	+	+	+/-
Positive p35S+tNOS+pFMV+pat	+	+	+	+	-	+	+/-
Positive tNOS+pFMV+pat+bar	-	+	+	+	+	+	+/-
Negative	-	-	-	-	-	+	+/-
Inconclusive [7,8]	+/-	+/-	+/-	+/-	+/-	-	+/-
Inconclusive [9]	+	-	-	-	-	+	-

[7] PCR inhibitions may be due to the presence of excessive DNA and/or PCR inhibitors. It is recommended to dilute the DNA extracted from the sample 1:10 or 1:100 in DNAse/RNAse free water and repeat the Real-Time PCR reaction. When applicable, the LOD of the method should be adjusted in accordance with the dilution factor.

[8] The appearance and characteristics of the amplification curves should be thoroughly considered. Incomplete amplification curves often denote low amount of DNA template. In this case, the positivity of the result is dubious, and the Real-Time PCR reaction should be repeated using a superior amount of DNA template.

[9] When exclusively the CaMV35S promoter gene is detected in the sample, the potential presence of the natural Cauliflower mosaic virus (CaMV) should be analysed using the InviScreen® CaMV Detection Kit (ref. 6014003201). The CaMV 35S promoter gene comes from the CaMV virus which naturally infects plant species. Plants naturally infected with the virus that test positive for the CaMV 35S promoter cannot be implicitly identified as GMOs. It is therefore important to differentiate between food products that effectively contain GMOs and non-GMO products naturally infected with the CaMV virus.

[10] Plant target as an endogenous control should be considered positive when Ct ≤ 35.

8. Quality Control

The test can only be considered valid under the following control conditions:

CONTROLS	Target DNA	Plant	IAC
Positive Control	+	+	+
Negative Control	-	+/-	+

If no amplification is observed for the positive control, the test results are invalid and must be repeated. The positive control template is expected to amplify before Ct 35. If amplification is observed for the negative control it indicates that the reagents have become contaminated while setting up the run, invalidating test results.

9. Performance Characteristics

Specificity: InviScreen® GMO Detection Kit (35S/NOS/FMV/pat/bar) was designed to specifically detect GMOs. The following reference materials (table below) were tested according to the general assay instructions with 100% agreement with the expected results.

Detection Limit and sensitivity: The limit of detection (LOD) is often matrix dependent, and the sensitivity of the analysis may be reduced depending on the total DNA extracted from the actual ingredient in test, but also its quality. This way, the LOD needs to be determined through in-house validation.

The detection limit of the method was determined based on the certified reference materials Maize MON89034 AOCS 0906E, Bt-11 Maize powder ERM®-BF412f and Bt-176 Maize powder ERM®-BF411f. Ten independent assays were performed with sequential dilutions down to 0,01% GMO, with 2 replicates per dilution. The LOD was determined as 0.1%.

Repeatability and Reproducibility: InviScreen® kits demonstrated to have an excellent dynamic range, with relative standard deviation of Ct values less than 5% for all tested concentrations.

Robustness: The method revealed to be highly reliable, and unaffected by small variations deliberately introduced. The obtained results from at least 10 independent experiments, performed in duplicate, were concurring and the expected outcome achieved.

Trueness: Trueness of the method proved to be of 100%, has the obtained results from at least 10 independent experiments, performed in duplicate, and were concurring with the expected outcome.

Reference material	CaMV35S promoter		NOS terminator		FMV promoter		pat		bar		Agreement
	Result	Expected	Result	Expected	Result	Expected	Result	Expected	Result	Expected	
1507 - 10% GMO	+	+	-	-	-	-	+	+	-	-	100%
Bt-11 - 5% GMO	+	+	+	+	-	-	+	+	-	-	100%
Bt-176 - 5% GMO	+	+	-	-	-	-	-	-	+	+	100%
GA21 - 4,4% GMO	-	-	+	+	-	-	-	-	-	-	100%
GTS 40-3-2 – 0,1% GMO	+	+	+	+	-	-	-	-	-	-	100%
GTS 40-3-2 – 10% GMO	+	+	+	+	-	-	-	-	-	-	100%
GTS 40-3-2 - GMO free	-	-	-	-	-	-	-	-	-	-	100%
MON810 - 10% GMO	+	+	-	-	-	-	-	-	-	-	100%
MON810 - 5% GMO	+	+	-	-	-	-	-	-	-	-	100%
MON88017 - 100% GMO	+	+	+	+	-	-	-	-	-	-	100%
MON89034 - 100% GMO	+	+	+	+	+	+	-	-	-	-	100%
NK603 - 0.1% GMO	+	+	+	+	-	-	-	-	-	-	100%
NK603 - 0.5% GMO	+	+	+	+	-	-	-	-	-	-	100%
NK603 - 1% GMO	+	+	+	+	-	-	-	-	-	-	100%