Instructions for use
InviScreen® CaMV Detection Kit

INVITEK diagnostics

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REF 6014003201 | Σ 50 tests | -25 °C / -15 °C | RUO

1. Intended use

InviScreen® CaMV Detection Kit detects cauliflower mosaic virus (CaMV) DNA in food samples. This PCR kit is intended for trained laboratory professionals and confirms the presence of a common GMO construct derived from CaMV. Positive results for the 35S promoter cannot be automatically identified as GMOs in plants naturally infected with viruses or samples contaminated with virus-bearing plant material. Such cases may result in false positive GMO identification. Therefore, it is crucial to distinguish between food products containing GMOs and non-GMO products naturally infected with CaMV. To avoid reporting false positives, it is important to report the detection of CaMV virus whenever the CaMV 35S promoter is exclusively found in the analyzed samples. This kit should be used in combination with InviScreen® GMO Detection kits.

2. Product description

InviScreen® CaMV Detection Kit provides a real-time PCR method for the detection of CaMV virus DNA. This real-time PCR assay was developed to detect the ORFIII region of the CaMV genome. The method relies on the amplification of CaMV DNA based on Taqman technology. The real-time PCR method can be performed in commonly available PCR instruments with a FAM fluorescence channel. 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).

3. Kit contents

REF.	COMPONENT	FUNCTION	CAP COLOR	QUANTITY
D24.01	Primer/Probe Mix[1]	Targeted detection		1 tube, 500 µL
D24.02	qPCR Master Mix[1]	Amplification		1 tube, 500 µL
D24.03	Negative Control	Negative Control		1 tube, 50 µL
D24.04	Positive Control	Positive Control		1 tube, 50 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 (optional) [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.

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 with the manufacturer instructions of the total genomic DNA extraction kit in use.

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 a reaction mix according to the table below:

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

Alternatively, the user may choose to prepare a working mix by mixing the total volume of the Primer/Probe Mix with the qPCR Master Mix in a separate tube (not provided). Vortex the mix gently and spin it down to obtain a working mix enough for 50 reactions. It is recommended to label the vial corresponding to the working mix.

1. Homogenize the reaction mixture 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 sec	45
3	Hybridization/extension plate reading *	59 °C	1 min	

* Fluorescence data must be obtained during this step through FAM (target DNA).

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 channel. After setting the threshold baseline, the analysis outcome should be interpreted according to the scenarios referred bellow.

A result is considered positive when Ct ≤ 35.

RESULT	Target DNA (FAM)
Positive	+
Negative	-
Inconclusive [6]	?

[6] 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.

8. Quality Control

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

CONTROLS	Target DNA (FAM)
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

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 method LOD was determined using p4lasmid construct containing the target gene, in 11 independent assays. The LOD was determined to be 10 copies/µl.