

Instructions for use

InviScreen® Mustard Detection Kit

INVITEK
diagnostics

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REF 6012008200

Σ 2 x 100 tests

-15 °C
-25 °C

RUO

1. Intended use

InviScreen® Mustard Detection Kit is intended for the qualitative detection of white mustard (*Sinapis alba*) and black/brown mustard (*Brassica nigra*) DNA in food and feed samples. According to Regulation (EU) No. 1169/2011, mustard is an allergenic substance which can cause immunological reactions in allergic or sensitive individuals. Since even traces of a given allergen can cause allergic reactions, clear labeling of foods is required by the EU to enable consumers to be informed and make their dietary decisions with safety. The kit is designed for use by trained professionals in laboratory settings to assist food and feed industry professionals in monitoring and controlling the presence of mustard allergens in food and feed products. The user assumes all responsibility for compliance with local regulations and guidelines for the use of this product. The kit is suitable for use in the detection of mustard DNA in raw materials, finished products, and environmental samples. This kit is not designed to quantify the level of mustard allergen present in a sample. The InviScreen® Mustard Detection Kit is intended for research use only and should not be used for diagnostic or therapeutic purposes.

2. Product description

InviScreen® mustard Detection Kit provides a real-time PCR method for the qualitative detection of white mustard (*Sinapis alba*) and black/brown mustard (*Brassica nigra*) DNA in food, feed, and environmental samples. The assay is based on the amplification of species-specific DNA using hydrolysis probes, which can be performed with a variety of food matrices. The kit allows simultaneous amplification of the allergenic target species and an internal amplification control (IAC) to exclude false negative results due to PCR inhibition. This duplex detection system helps maximize amplification accuracy and simplify reaction setup. This 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 10 mg allergenic substance/kg food sample.

3. Kit contents

REF.	COMPONENT	FUNCTION	CAP COLOR	QUANTITY
D13.01	Primer/Probe Mix - White Mustard ¹	Targeted detection	●	1 tube, 1000 µL
D13.02	Primer/Probe Mix - Black Mustard ¹	Targeted detection	●	1 tube, 1000 µL
D13.03	qPCR Master Mix ¹	Amplification	●	2 tubes, 1000 µL
D13.04	Negative Control	Negative Control	●	1 tube, 200 µL
D13.05	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 (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 To ensure accurate analysis of samples with multiple ingredients that are not in a powdered or granulated form, it is recommended to homogenize them carefully.

³ The assay was validated on a Bio-Rad CFX96 instrument.

⁴ It is recommended to quantify and assess the purity of the extracted 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 PCR analysis 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 case of surface sampling, it is advised to perform sample collection using pre-moistened swabs in distilled water or sterile saline solution and rub the test surface (100 cm² area). 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 two reaction mixes (one for each Mustard species) 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 100 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	5 min	1
2 Denaturation	95 °C	20 seg	50
3 Hybridization/extension plate reading *	60 °C	1 min	

* Fluorescence data must be obtained during this step through FAM (target DNA) and HEX/VIC (internal amplification control) channels.

Instructions for use

InviScreen® Mustard Detection Kit

c. Results interpretation

The results should be interpreted in accordance to 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 and the amplification control in HEX/VIC channel. After setting the threshold baseline, the analysis outcome should be interpreted according to the scenarios referred below.

The reaction includes an internal amplification control to monitor the reaction and to exclude the occurrence of PCR inhibition. A poor signal indicates the presence of PCR inhibitors in the reaction which compromises an effective and accurate detection test. Under optimal conditions, the lowest amount of mustard DNA detected in 100% of the experiments was at least 10 mg/kg of allergenic species DNA with respect to reference material.

A result is considered positive when $Ct \leq 45$.

RESULT	Target DNA (FAM)	IAC (HEX/VIC)
Positive	+	+/-
Negative	-	+
Inconclusive ^{6,7}	-/?	-

⁶ PCR inhibition 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 to the dilution factor.

⁷ The appearance and characteristics of the amplification curves should be thoroughly considered. Incomplete amplification curves often indicate 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)	IAC (HEX/VIC)
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 30.

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® Mustard Detection Kit was designed to specifically detect mustard species. The following DNA extracts (Table below) were tested according to the general assay instructions and no amplification was obtained for species other than mustard (highlighted in **Bold**).

MEAT

Chicken (*Gallus gallus*)
Cow (*Bos Taurus*)
Pig (*Sus scrofa domestica*)

FISH AND SEAFOOD

Codfish (*Gadus Morhua*)
Shrimp (*Peaneus Vannamei*)

FRUITS AND VEGETABLES

Almond (*Prunus dulcis*)
Black/Brown Mustard (*Brassica nigra/juncea*)
Brazil Nut (*Bertholletia excelsa*)
Cashew (*Anacardium occidentale*)
Celery (*Apium graveolens*)
Hazelnut (*Corylus avellana*)
Linseed (*Linum usitatissimum*)
Lupine (*Lupinus albus*)
Macadamia nut (*Macadamia integrifolia*)
White pepper (*Piper nigrum*)
Maize (*Zea mays*)
Mustard (*Sinapis alba*)
Nutmeg (*Myristica fragrans*)

Oats (*Avena sativa*)
Peanut (*Arachis hypogaea*)
Pecan nut (*Carya illinoensis*)
Pine nut (*Pinus pinea*) Pistachio (*Pistacia vera*)
Rice (*Oryza sativa*)
Rye (*Secale cereal*)
Sesame (*Sesamum indicum*)
Soy (*Glycine max*)
Sunflower (*Helianthus annuus*)
Tomato (*Solanum lycopersicum*)
Walnut (*Juglans regia*)
Wheat (*Triticum aestivum*)

Detection limit and sensitivity: The limit of detection (LOD) for a given analysis is often influenced by the sample matrix and the quantity and quality of the DNA extracted from the target ingredient. To determine the LOD for a specific method, in-house validation experiments are necessary. In our study, the LOD was determined through ten independent experiments that evaluated the sensitivity of the method. Internal DNA standards with decreasing amounts of mustard DNA were used in the validation, either as pure genomic DNA or spiked matrices. Under optimal conditions, the method was able to detect at least 15 pg of mustard DNA or 10 ppm.

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 with the expected outcome.

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

Performance characteristics for validation: To evaluate the false positive and false negative rates, a total of 306 samples were tested in independent experiments, with 199 samples known to be positive and 107 samples known to be negative. Each experiment was performed at least twice. Our method showed no false positive or false negative results, with both rates at 0%, resulting in 100% positive predicted value (PPV) and negative predicted value (NPV).