ENCODE pFMV IDENTIFICATION KIT

ENCODE INNOVATIONS LLP. B1- Ground Floor, Gayatri Res . Opp. Khandagle estate PURNA, Bhiwandi Thane (M.H.)421302 cont.: 9920967965 Email : encodeinnovationsllp@gmail.com LLP Identification Number:ABA-3123

ENCODE p-FMV IDENTIFICATION KIT

ENCODE pFMV ID Kit Cat .No. EIL-pFMV-10
ENCODE pFMV ID Kit Cat .No. EIL-pFMV-25
ENCODE pFMV ID Kit Cat .No. EIL-pFMV-50
ENCODE pFMV ID Kit Cat .No. EIL-pFMV-100

TABLE OF CONTENTS

1. Product description
2. Technology description
3. Application
4. Contents and storage
5. Additional material required
6. Equipment required
7. Precautions and recommendations
8. Procedure
9. Specificity
10. Sensitivity
11. References
12. Additional information

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 organisms or plants.
Genetically modified (GM) 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 (1). In most countries, such use is highly regulated with 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 (2). 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 (3). Such qualitative screening methods provide a presence/absence response and help reducing the number of subsequent identification analyses. Although 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, these regulatory elements do not cover such important transgenic events as MON89788 soy, H7-1 sugar beet or GT73 rape. Therefore, in order to ensure a widest screening, it is important to also detect a promotor from Figwort Mosaic Virus (P-FMV) and real-time PCR methods have proved to be a reliable strategy to perform this screening.

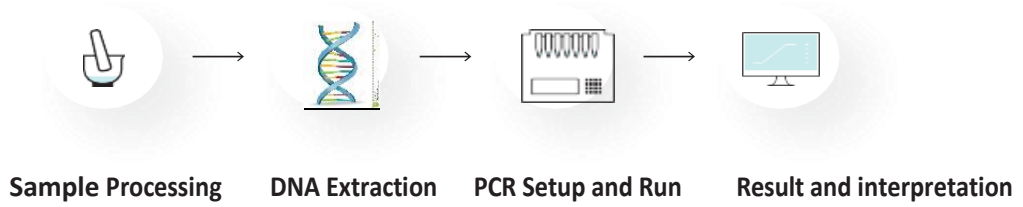
2. TECHNOLOGY DESCRIPTION

PCR is a method used to amplify a specific DNA sequence which is typically amplified in a reaction containing a thermostable DNA polymerase, nucleotides, and primers complementary to the target sequence. When this solution is heated, the DNA molecule denatures, 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, specific fluorescent probes are used to detect the amplified DNA

by hybridizing with amplicons. These probes are linked to a fluorophore on one end and a quencher that suppresses fluorescence on the other. If the target sequence is present during the PCR, amplification occurs and the probe is degraded, resulting in fluorescence increase. Fluorescence is measured by a detector and the associated software plots the fluorescence intensity versus number of cycles, allowing the determination of the presence or absence of the target organism.

3. APPLICATION

This test allows the detection P-FMV elements in food products after DNA extraction. It can also be used with animal feed" and other samples in which GMO detection is needed. The specific DNA probes are detected in FAM channel. The entire procedure includes the following main steps:



4. KIT CONTENTS AND STORAGE

TABLE :1 The kit contains and Reaction Size available as below

Reagents	10 reactions	25 reactions	50 reactions	100 reactions
PCR MIX	125 µl	312.5 µl	625 µl	1250 µl
PFMV detection mix	25 µl	62.5 µl	125 µl	250 µl
PCR grade water	80 µl	200 µl	400 µl	800 µl
Negative control	20 µl	50 µl	100 µl	200 µl
pFMV Positive control	5 µl	10 µl	20 µl	40 µl

Store all contents at -20ºC and protected 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 on the tube.

5. ADDITIONAL MATERIAL REQUIRED

Disposable powder-free gloves
DNA extraction kit
Sterile pipette tips with filters
Tubes/Strips and accessories specific for the Real Time PCR Instrument

6. EQUIPMENT REQUIRD

Laminar Air Flow Cabinets/PCR Cabinets
Micropipettes
Microcentrifuge
Real Time PCR instrument
accessories Freezer, refrigerator

7. PRECAUTIONS AND RECOMMENDATIONS

The use of REAL TIME DETECTION KIT P-FMV involves PCR amplification.
The kit provides all reagents required for the PCR. In order to get reliable results, the entire assay shall be performed in conditions that avoid nuclease carry over or DNA cross contamination:

- Prepare appropriate aliquots of the solutions, keep them separated from other reagents in the laboratory and use them rather these aliquots directly pipetting from stock solutions.
- Use nuclease-free labware (e.g. pipettes, pipette tips, reaction vials).
- Wear gloves when performing the assay.
- To avoid cross-contamination of samples and reagents, use fresh aerosol-preventive pipette tips.
- Add the positive control after closing all tubes.
- If possible, physically separate the workplaces for DNA preparation.

8. PROCEDURE

8.1 Sample preparation

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

8.2 DNA extraction

Use a kit or protocol suitable for DNA extraction from food products. Follow the manufacturer's or authors instructions.

8.3 PCR preparation A - PCR mix

Always wear gloves for all PCR procedures.

- Thaw the kit solutions and briefly spin vials before opening to ensure the complete recovery of volumes. Mix carefully but thoroughly by pipetting up and down.

To prepare 25 µl standard reactions, prepare the PCR Mix by adding the following volumes of kit components:
Note: the volumes indicated below are based on a single standard reaction. Prepare the appropriate mix volume by multiplying the amounts indicated by the number of reactions to be performed (including a positive and a negative control) plus one or two reactions to cover pipetting losses.

TABLE :2

Reagent	Volume per reaction (25 µl)
Mater mix xl	12.5 µl
P-FMV Detection mix	2.5µl
Sample /Template DNA	2 µl
PCR Grade water	8 µl
Total volume	**25µl**

a) Mix carefully but thoroughly by pipetting up and down (do not vortex).

b) Briefly spin sample tubes before opening to prevent cross contamination.
c) In separate tubes add reagents as per **table 2**
d) Close the PCR tubes and briefly spin in a suitable centrifuge.
e) Place the reactions into the Real Time PCR instrument

8.4 Program set up

Prepare the real time PCR instrument according to the following temperature/time program:

Stage	Time	Temp	No of cycles
Initial denaturation	95°c	15 min	1
Denaturation	95°c	15 sec	
Annealing and Extension	60°c	60 sec	
Denaturing, Annealing and Extension			40

Passive reference dye: - ROX
** If Needed, prepare a fresh dilution of ROX internal reference dye and use **0.3 µl** for each reaction. The diluted ROX reference dye must be kept in a light-protected tube at 4°c

- **For High ROX** compatible instrument dilute 5 µl ROX in 50 µl PCR Grade Water (1:10).
- **For Low ROX** compatible instrument dilute 0.5 µl ROX in 50 µl PCR Grade Water (1:100).

8.5 Data interpretation

Probe for P-FMV detection are labeled with FAM and must be analyzed in the corresponding fluorescence channel.

A) CONTROLS

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

Negative control	Positive control	Interpretation
-	+	perfect
-	-	Repeat the experiment, pipetting error
+	+	Contamination, repeat the experiment
+	-	Repeat the experiment, pipetting error

Note: - 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:

pFMV detection FAM	IC (internal control)	Interpretation
+	+	FMV detected (positive)
-	+	FMV not detected (negative)
-	-	PCR inhibition, poor extraction
+	-	PCR inhibition, repeat the experiment

Note: - When both P-FMV and IC is not detected, the sample must be tested again after 1:10 dilution.

9. SPECIFICITY

100% Exclusivity, determined using DNA from non-target GMOs and other vegetables suitable to occur in the same food products.

10. SENSITIVITY

A detection limit of 250 pg of target DNA can be achieved ENCODE pFMV IDENTIFICATION KIT. About 0.5% of the target GMO can be detected in food samples when using 100 ng of total DNA.

11. REFERENCES

http://cera-gmc.org
http://gmo-crl.jrc.ec.europa.eu/gmomethods

12. ADDITIONAL INFORMATION

For questions contact Email: encodeiinovationsllp@gmail.com
Ms. Tithi Patel - 9970004798