Xpert qDetect P-35S, T-NOS and P-FMV

#GDK21.0150 (50 rxns each)
(FOR RESEARCH ONLY)

Product: Since 1994, over 100 genetically modified plants have received approval for use as food or animal feedstuff. In most countries, usage of GMOs is highly regulated. For the enforcement of national legislation, efficient detection of genetically modified organisms in food and feed products is essential. Taking into account the large diversity, an initial generic screening for the presence of the most common GMO material is usually the first step in GMO analysis, in order to reduce the amount of subsequent identification analysis. As the promotor 35S from cauliflower mosaic virus (CaMV) and the terminator NOS from *Agrobacterium tumefaciens* are the most frequent elements present in transgenic material found in food and feed, detection of these regulatory sequences by PCR/qPCR amplification is the most logical choice. However, since these do not cover some of the important GMOs such as MON89788 Soy, H7-1 sugar beet or GT73 oilseed rape, to ensure the widest screening, this kit is also suitable for the detection of the promotor from FigWort Mosaic Virus (P-FMV) by PCR/qPCR.

The Xpert qDetect P-35S, T-NOS and P-FMV is a kit that allows for the detection by qPCR of DNA sequences from the promotor 35S and/or the terminator NOS and/or the promotor FMV present in total DNA previously purified from food or feed samples. This kit is compatible with instruments equipped with FAM and ROX channels. The detection limit is 10-100 pg of GMO DNA, allowing the detection of as little as 0.01-0.1% of target DNA in food samples if 100ng of total DNA (mixed species) is used

Applications: Detection of GMO material in food and feed.

Contents: The qPCR Detection Kit (#GDK21.0150) for P-35S, T-NOS and P-FMV contains sufficient reagents for 50 qPCR reactions for each of the 3 targets.

Component	GDK20.0200
GMO Mix A	3x 840 µl
P-35S Mix	105 µl
T-NOS Mix	105 µl
P-FMV Mix	105 µl
Positive Control (GMO+)	105 µl
Negative Control (GMO-)	105 µl

Note: This product does not include reagents and other materials required for DNA extraction.

Samples: 2µl of DNA (previously purified from food products using commercially available DNA purification kits according to manufacturer´s instructions or author´s instructions).

Properties: Fast, Easy and Reliable
Low limit of Detection
100% Specificity, determined using DNA from 10 vegetable species and 8 to 19 non-target GMOs (depending on the target).
Compatible with instruments equipped with **FAM** and **ROX** channels

Storage: -20ºC and protected from light for at least 1 year. Minimize repeated freeze/thawing, consider preparation of aliquots.

Prior to use:

General Considerations

In order to prevent erroneous results due to contamination and/or degradation of DNA, it is strongly recommended to have dedicated materials, equipment and area for the DNA extraction, separated from the preparation of PCR procedures. Workflow in the laboratory should be unidirectional, starting with DNA isolation and moving to preparation of PCR amplification and finally to (q)PCR and Detection. Moreover, all molecular biology procedures, including DNA extraction, require qualified personnel to prevent risk of erroneous results and/or inadequate interpretation of results. Disposable gloves must be worn during all procedures. All relevant National and Local Regulations must be met.

Food Sample Preparation

Representative food samples should be prepared conform standard procedures and under GLP conditions.

DNA extraction

DNA is extracted using an appropriate kit (not included) for the extraction of DNA from food samples using the manufacturer´s instructions and/or according to literature following author´s instructions.

Signal Detection

Unambiguous detection of GMO DNA (and of the internal control) requires a suitable calibration of both FAM and ROX channel. Please refer to the manufacturer´s instruction of the real time PCR cycler.

qDetect - Basic Protocol

1. Mix for each qPCR reaction:

Component	Volume
GMO Mix A	16 µl
P-35S Mix **or** T-NOS mix **or** P-FMV Mix	2 µl

In order to minimize risk of contamination, reagent loss and improve pipetting accuracy, we recommend to prepare a mastermix for multiple samples (N), always including a negative control, and a positive control, by mixing all components (N+2), except template DNA (nor control DNA), dividing the mixture equally into each PCR tube (18 µl each), briefly spin tubes (or tap down) and then add 2 µl template DNA or control DNA directly in the mixture.

2. Set-up qPCR cycling:

Nº cycles	Temp	Time	Acquisition
1x	50ºC	2 min	No
1x	95ºC	5 min	No
45x	95ºC	30 sec	No
	60ºC	30 sec	Yes
	72ºC	30 sec	No

After an initial cycle of 2 min at 50ºC and 5 min at 95ºC (Enzyme activation and denaturation of template), cycle 45 times for 30 seconds at 95ºC, 30 seconds at 60ºC and 30 seconds at 72ºC. Acquire data for the detection of GMO DNA on the **FAM** channel. Probe to detect specific amplification of the internal control, which is included in GMO Mix A and which is amplified simultaneously with the target DNA, should be detected in the **ROX** channel.

Results

Controls

In order to validate the assay, controls must have the following results. If the signal of one of the controls does not match, the whole experiment, including all samples, must be repeated.

Control	FAM channel	ROX channel
Negative Control	Ct=N/A	positive
Positive Control	positive	unimportant*

N/A = Not applicable (signal below threshold).

*unimportant: is expected to be positive, however, if negative but other controls match expected results, this makes no difference

Samples

For each sample, there are 4 possible outcomes, as summarized in the table below.

FAM channel	ROX channel	Result
positive	positive	positive
positive	Ct=N/A	positive
Ct=N/A	positive	negative
Ct=N/A	Ct=N/A	inhibition*

*) in case both GMO (FAM channel) and Internal Control (ROX channel) have signals below threshold, but all the controls resulted in signals as expected, the sample must be retested, as the qPCR reaction was inhibited. Inhibition often is the result of a too high DNA concentration and therefore it is recommended that retesting should be carried out with a 10-fold dilution of the original DNA sample.