GENERON
Advanced Transfer Technologies

# **MODIfinder** DETECTION ASSAY GMO COTTON

## User Guide

# 1 - Introduction

*The introduction of GMO crops in the food chain led to the need to investigate their presence in a wide range of raw materials, semi finished and finished products as well as in animal feed.*

*The European Commission first introduced thresholds for the accidental unavoidable presence of GM ingredients; and according to EU Reg. CE/1829/2003 all products (food, feed, GMO derivatives, cultures, aromas and additives) are labeled as to their GMO content.*

*Real-Time PCR in time established as the gold-standard method for GMOs detection and specific ISO norms (namely ISO21568-21569-21570-21571) regulate it's usage. The base for GMO COTTON methods is the detection of DNA sequences of genetic control elements such as promoters, transcription terminators, and markers, such as resistance genes.*

**Assays performances**

The MODIfinder GMO COTTON Detection Assay will detect the GMO only if all of the recommended components are stored properly and the recommended protocols are followed.

When used along with Generon ION Force DNA Extractor FAST (Cat. N. EXD001) the assay shows a Limit of Detection of 0.01%.

Detection limit is strictly dependent from the matrix and the genome size of the taxa under investigation, i.e. there is a theoretical LOD you cannot go below.

**Assays available**

| Part number | |
|---|---|
| PGT01A | MODIfinder COTTON MON531/757/1076 (BOLLGARD) |
| PGT02A | MODIfinder COTTON MON1445/1698 |
| PGT03A | MODIfinder COTTON 281-24-236 |
| PGT04A | MODIfinder COTTON 3006-210-23 |
| PGT05A | MODIfinder COTTON LibertyLink LL25 |
| PGT06A | MODIfinder COTTON MON15985 |
| PGT07A | MODIfinder COTTON GHB614 |
| PGT08A | MODIfinder COTTON MON88913 |
| PGT09A | MODIfinder COTTON GHB119 |
| PGT10A | MODIfinder COTTON T304-40 |

# 2 - MODIfinder GMO COTTON Detection Assay

When used along with GENERase Mastermix (Cat. N. ENG001) this Real-Time PCR assay detects a specific DNA sequence in the DNA of target in less than 1.5 hours. The amplification of the target sequence is measured by the use of a specific fluorescence-labeled probe (FAM).

## 2.1 - Assay Content

| | Box 50 reactions | | Box 100 reactions | |
|---|---|---|---|---|
| | N. vials | Volume (µl) | N. vials | Volume (µl) |
| MODIfinder OLIGO Mix * (OLIGOS and Probe pre-blended mix) | 1 | 150 | 2 | 150 |
| Positive Control | 1 | 120 | 2 | 120 |
| Negative Control | 1 | 1000 | 1 | 1000 |

** reagents are supplied with a 5% of extra volume.*

## 2.2 - Storage & Expiry information

Expiry date: see date on the packaging, product validity refers to the product kept intact in its original packaging. Protect reagents from light exposure as far as OLIGO Mix reagents are photosensitive. Store frozen.

# 3 – Materials and equipments needed

## 3.1 – Extraction[1]

| Material/Equipment | Source |
|---|---|
| Extraction Kit | Generon ION Force DNA extractor FAST (Cat. N. EXD001) |
| Chemicals: n-esane | Lab Suppliers |
| Tubes, 50 ml and 15 ml | Generon or other Lab Suppliers |
| DNAse/RNAse Free Water | Generon or other Lab Suppliers |
| Vortexer | Generon or other Lab Suppliers |
| Benchtop Centrifuge for 50 ml Tubes | Generon or other Lab Suppliers |
| Thermal Water Bath or Block | Generon or other Lab Suppliers |
| Pipette sets | Generon or other Lab Suppliers |
| Pipette tips (Barrier) | Generon or other Lab Suppliers |
| Tube rack for 1.5 ml tubes | Generon or other Lab Suppliers |
| 2.0 and 1.5 ml micro-tubes | Generon or other Lab Suppliers |
| Micro centrifuge for 1.5-2.0 ml micro-tubes | Generon or other Lab Suppliers |
| DNA Extraction VACUUM BOX + Vacuum pump or Venturi meter | Generon or other Lab Suppliers |

Each step of sample preparation (grinding, transferring, weighing, etc.) must be done according to GLP so that chance of cross-contamination between samples is minimized. It is recommended to use disposable equipment when possible.
If the food samples are not in a powdered or granular form, they should be processed (grinded or blended) before DNA extraction. The majority of DNA extraction methods supports from 20 to 50 mg of starting material. Generon ION Force DNA Extractor FAST (Cat. N. EXD001) allows processing up to 20 grams of starting material in order to maximize sample's lot representation.
Once the sample has been pulverized/homogenized, it can be weighed and the appropriate amount extracted according to DNA extraction method selected. Refer to manufacturer user manual for extraction procedure details.

## 3.3 – Detection via Real-Time PCR

| Material/Equipment | Source |
|---|---|
| Real-Time PCR System [2] | Generon or other Lab Suppliers |
| MODIfinder GMO COTTON Detection Assay | Generon (Cat. N. PGTxxA) |
| GENERase Mastermix | Generon (Cat. N. ENG001) |
| Optical Adhesive Seal and Optical reaction plate or Optical Caps and Strips | Generon or other Lab Suppliers |
| Micropipette sets | Generon or other Lab Suppliers |

(1) Equipment necessary only when ION Force DNA Extractor FAST (Cat. N. EXD001) is used.
(2) The assay can be used with Biorad CFX and MiniOpticon, Stratagene MxSeries, ABI 7300-7500-7900-StepONE-StepONE Plus, Light Cycler 480, Eppendorf realplex, Rotor-Gene Q etc. The assay is not compatible with Roche Light Cycler I and II.

# 4 – Real-Time PCR detection

## 4.1 – Reaction setup

I. Allow the reagents to thaw (GENERase Mastermix, MODIfinder OLIGO MIX, Positive Control and Negative Control). Vortex tubes when thawed and spin to collect contents at the bottom of the vial.

II. Mix 150 µl of MODIfinder OLIGO Mix with 750 µl of GENERase Mastermix to prepare MODIfinder Working Mastermix (WMX).

III. Vortex briefly and spin down in order to homogenize the mix.

IV. Transfer 18 µl of WMX into each well.

V. Add 12 µl of Negative Control into wells acting as negative control.

VI. Add 12 µl of each sample into wells testing the unknown samples.

VII. Add 12 µl of Positive Control into wells acting as positive control.

VIII. Close wells and ensure no bubbles are present at the bottom of the wells.

IX. Spin briefly optical PCR tubes or plates.

## 4.2 – Instrument setup

With GENERase Mastermix set the following parameters on your thermocycler:

I. Total Reaction volume: 30 µl

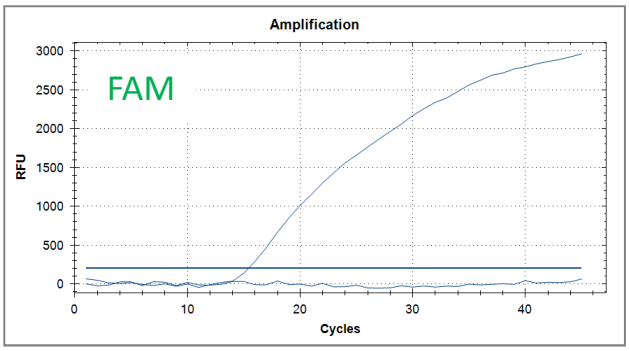
II. Fluorophores/Quenchers: Target GMO COTTON (FAM/BHQ1-NFQ);

III. Thermal profile:

| Step | T (°C) | Duration | Loops |
|---|---|---|---|
| UNG | 50 | 2 min | 1 |
| Taq Activation | 95 | 10 min | 1 |
| DNA Denaturation | 95 | 15 sec | 45 |
| Annealing/Extension + Plate Reading | 60 | 60 sec | |

# 5 – Data Interpretation

Results evaluation must be done according to the analysis software recommended by the Real-Time PCR instrument manufacturer. After performing PCR, each individual sample is analyzed through the instrument software to produce a Cq value (quantification cycle) for each reporter dye. These values are used to determine the presence (Qualitative Test) of GMO cotton into the sample. See below an example of the graphics obtained for a positive (Fig. A) control for the GMO cotton target amplification (blue line).




After setting the baseline, the analysis outcome should be evaluated following the indications below.

If the following conditions are met:

| TEST | GMO COTTON (FAM ) |
|---|---|
| Positive Control | + |
| Negative Control | - |

Then the possible results for any sample are:

| TEST | GMO COTTON (FAM ) |
|---|---|
| Positive Sample | + |
| Negative Sample | - |
| Invalid Sample (Inhibited) | - |

In case of inhibition DNA isolation and purification for the sample need to be improved or you may need to dilute your sample before performing a new test. Refer to the Troubleshooting paragraph (section 6) for further suggestions.

# 6 – Troubleshooting

I. Concomitant no target or endo amplification, or amplification plots grossly abnormal. Possible causes and corrective actions:

- An excess of DNA in the target might inhibit the reaction and ENDO may be affected due to an excess of DNA and/or PCR inhibitors. Test samples diluted 1:10 and 1:100. Please, use DNase/RNase Free Water to prepare dilutions.
- Inadequate sealing of optical caps/film caused sample evaporation. Redo the analysis using proper tools and proper optical caps/film to secure perfect sealing.
- Did not use the proper consumables. Redo the analysis and use only optical grade 96-well plates and optical adhesive seal or optical 8-well strips and caps.
- Samples were not properly prepared. Remake the sample DNA preps. Ensure that the DNA extraction method is properly performed.

II. Positive Control reactions failed to amplify, but other reactions appear correct (e.g. the endo is amplified):

- Positive Control DNA was not added to the reaction wells. If other reactions look normal, there may be no need to repeat the run.

III. Negative Control reactions are positive:

- Contamination of the negative control vial or the MODIfinder PCR mix with MODIfinder-positive DNA. Use more care to prevent contamination while handling assay reagents and setting up assays.

In case support is needed contact Generon at: support@generon.it

# 7 – Disclaimers

The product is intended for research use only. Generon makes no warranty of any kind, either expressed or implied, except that the materials from which its products are made of standard quality. If any materials are defective, Generon will provide a replacement product. Generon shall not be liable for any damages, including special or consequential damage, or expense arising directly or indirectly from the use of this product. Please do not interchange components between assays of different lot numbers. This assay is designed to be used by laboratory personnel following the common molecular biology precautions.

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| | |
|---|---|
| Product Line: | MODIfinder |
| Type: | qualitative |
| Storage: | Frozen |
| Execution time: | about 120 minutes |
| Expiry date: | see date on the packaging, product validity refers to the product kept intact in its original packaging and constantly under suitable temperature conditions as mentioned above. |

## Assay Box Content

| | Box 50 reactions | | Box 100 reactions | |
|---|---|---|---|---|
| | N. vials | Volume (µl) | N. vials | Volume (µl) |
| MODIfinder OLIGO Mix * (OLIGOS and Probe pre-blended mix) | 1 | 150 | 2 | 150 |
| Positive Control | 1 | 120 | 2 | 120 |
| Negative Control | 1 | 1000 | 1 | 1000 |

* All reagents are supplied with a 5% of extra volume.

Not Provided Article: GENERase Mastermix (Cat. N. ENG001) or equivalent.

## Reaction Setup

Protect reagents from light exposure as far as OLIGO Mix reagents are photosensitive.

Before setting the analysis, we strongly advise to leave the reagents to warm up at room temperature. Vortex briefly OLIGO mix, afterwards spin to collect contents at the bottom of the vials. Spin GENERase Mastermix (Cat. N. ENG001) before opening it.

Prepare MODIfinder WORKING Mastermix by adding 150 µl of MODIfinder OLIGO Mix into each tube prefilled with 750 µl of GENERase Mastermix (Cat. N. ENG001) in order to obtain a single volume of 900 µl of MODIfinder WORKING Mastermix. Vortex briefly MODIfinder WORKING Mastermix with the aim of homogenizing the mix and excluding $MgCl_2$ gradient that could impair the results. Spin to collect contents at the bottom of the vial *(Note: label GENERase vials with target name after OLIGO Mix addition)*. Vortex briefly Positive Control and samples before proceeding further, spin to collect contents at the bottom of the vial.

Transfer MODIfinder WORKING Mastermix and samples into the plate as follows:

| Reagents per well | Volume |
|---|---|
| Unknown Sample | 12 µl |
| Positive Control<br>Negative Control | |
| MODIfinder WORKING Mastermix | 18 µl |
| Final Volume | 30 µl |

## Detector Setup

| Target | Reporter Dye | Quencher Dye |
|---|---|---|
| GMO COTTON | FAM | BHQ1-NFQ |

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## Thermal cycling

| Step | T (°C) | Duration | Loops |
|---|---|---|---|
| UNG | 50 | 2 min | 1 |
| Taq Activation | 95 | 10 min | 1 |
| DNA Denaturation | 95 | 15 sec | 45 |
| Annealing/Extension + Plate Reading | 60 | 60 sec | |

The thermal profile presented above was optimized for GENERase Mastermix (Cat. N. ENG001).

## Results analysis

If the following conditions are met:

| TEST | GMO COTTON (FAM ) |
|---|---|
| Positive Control | + |
| Negative Control | - |

Then the possible results for any sample are:

| TEST | GMO COTTON (FAM ) |
|---|---|
| Positive Sample | + |
| Negative Sample | - |
| Invalid Sample (Inhibited) | - |

In case of inhibition DNA isolation and purification for the sample need to be improved or you may need to dilute your sample before performing a new test. Refer to the Troubleshooting paragraph , section 6 in the User Guide, for further suggestions.

## Warning and Precaution

Please do not interchange components of assays with different lot numbers. This assay is designed to be used by laboratory personnel following the common molecular biology precautions (GLP).

## Disclaimer

Generon s.r.l. guarantees the buyer exclusively concerning the quality of reagents and of the components used to produce the Assay. Generon S.r.l. is not responsible and cannot anyway be considered responsible or jointly responsible for possible damages resulting from the utilization of the product by the user. The user consciously and under his own responsibilities decides for the utilization purposes of the product and uses it the way he considers most suitable in order to reach his goals and/or objectives.

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The product was internally tested by our quality control. Any responsibility is waivered if the warranty of quality control does not refer to the specific product. The user is personally responsible for data that he will obtained and/or he will supply to third parties using this assay. Once the sealed package is open the user accepts all the conditions without fail; if the package is still sealed the product can be returned and the user can be refunded.