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Advanced Transfer Technologies

MODIfinder QUANTITATIVE ASSAY GMO

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 QUANTITATIVE methods is the detection of DNA sequences of genetic control elements such as promoters, transcription terminators, and markers, such as resistance genes.

In a classic GMO Quantitative experiment a single DNA extract will be used as a template on two PCR systems: the first one detects **a GM-specific target DNA sequence (GM)**; the other is an **Endogenous Reference (ER)** system designed to serve as a quantitative reference detecting both genetically modified or non-genetically modified plant DNA. Results are then compared to calculate a %GMO figure for each sample.

MODIfinder Quantitative assay contain DNA from standard powders (Certified Reference Material-IRMM) pre-extracted using Generon ION Force DNA Extractor FAST (Cat. N. EXD001) called as Standard Points for both GM-fragment and ER-fragment detection.

Both GM- and ER- standard points must be analyzed on the same reaction plate of the unknown samples. Double GM- and ER- standard curves and two negative control reactions are recommended to be setup for each PCR run according with the good laboratory practice.

Assays performances

The MODIfinder GMO Quantitative 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 (LOD) 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	
PGExxQ	MODIfinder Generic Quantitative Assay
PGBxxQ	MODIfinder Sugar Beet GMO Quantitative Assay
PGCxxQ	MODIfinder Corn GMO Quantitative Assay
PGSxxQ	MODIfinder Soy GMO Quantitative Assay
PGTxxQ	MODIfinder Cotton GMO Quantitative Assay
PGPxxQ	MODIfinder Potato GMO Quantitative Assay
PGZxxQ	MODIfinder Rapeseed GMO Quantitative Assay
PGRxxQ	MODIfinder Rice GMO Quantitative Assay

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2 - MODIfinder GMO QUANTITATIVE

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 and the endo sequences 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 GM OLIGO Mix * (OLIGOS and Probe pre-blended mix)	1	150	2	150
MODIfinder ER OLIGO Mix * (OLIGOS and Probe pre-blended mix)	1	150	2	150
GM- Standard Points	5	120	5	120
ER- Standard Points	5	120	5	120
Negative Controls	2	1000	2	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 Quantitative Assay	Generon (Cat. N. PGxxxQ)
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

Allow the reagents to thaw (GENERase Mastermix, MODIfinder OLIGO MIX for the GM-target and for ER-target , 5 GM-Standard Points and 5 ER-Standard Points). Vortex tubes when thawed.

<u>Reaction set-up for GM-Target</u>

I. Mix 150 µL of MODIfinder GM-target OLIGO Mix with 750 µL of GENERase Mastermix to prepare MODIfinder Working Mastermix (WMX-GM) (label vials with GM-Target name).
II. Vortex shortly and spin down in order to homogenize the mix.
III. Transfer 18 µL of WMX-GM into PCR plate wells according to the number of unknown samples, number of GM-Standards Points and number of acting well as negative control.
IV. Add 12 µL of negative control into wells acting as negative control.
V. Add 12 µL of each sample to wells testing the unknown samples.
VI. Add 12 µL of GM-Standards Points to wells to create a standard curve for GM-target.

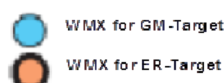
Close wells and ensure no bubbles are present at the bottom of the wells.

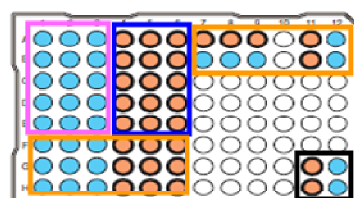
<u>Reaction set-up for ER- Target</u>

I. Mix 150 µL of MODIfinder ER-target OLIGO Mix with 750 µL of GENERase Mastermix to prepare MODIfinder Working Mastermix (WMX-ER) (label vials with ER-Target name).
II. Vortex shortly and spin down in order to homogenize the mix.
III. Transfer 18 µL of WMX-ER into other PCR plate wells according to the number of unknown samples, number of ER- Standards Points and number of acting well as negative control.
IV. Add 12 µL of negative control into wells acting as negative control.
V. Add 12 µL of each sample to wells testing the unknown samples.
VI. Add 12 µL of ER-Standards Points to wells to create a standard curve for ER-target.

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

PINK=Wells aimed for GM-STANDARD POINTS
BLUE=Wells aimed for ER-STANDARD POINTS
ORANGE=Wells aimed UNKNOWN SAMPLES
BLACK=Wells aimed for NEGATIVE CONTROL





E.g.: example of plate setup for a proper quantitative GMO analysis where either GM- and ER- standard points and unknown samples are in triplicates with a double negative control for both targets.

4.2 – Instrument setup

With GENERase Mastermix set the following parameters on your thermocycler:

I. Total Reaction volume: 30 µL
II. Fluorophores/Quenchers: GM- target and ER- target (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	

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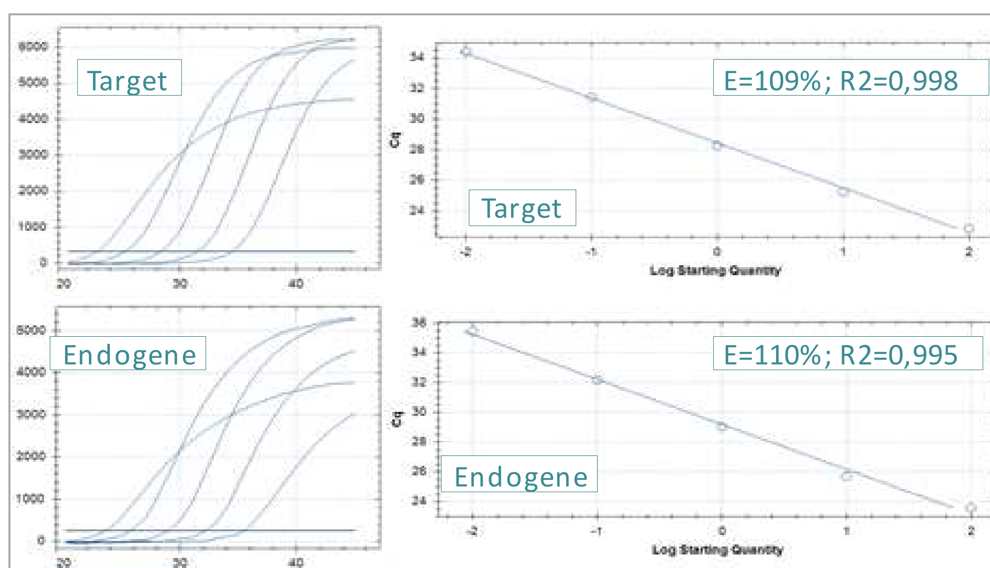
5 – Data Interpretation

Results evaluation must be done according to the analysis software recommended by the Real-Time PCR instrument manufacturer.

After running the samples on the instrument, data are analyzed using the software to produce Cq values of each reporter dyes for each sample run. These values are then used to determine the presence and, afterwards, quantify the amount of GMO material in each sample.

The amount of genetic modified material (GM-target) must be normalized to the amount of endogenous plant material (ER-target) detected in each sample. GM-target and ER-target Cq of *unknown samples* are interpolated on the respectively calibration/standard curve obtained through Cq values of the *GM-target concentration Standard Points* and *ER- target concentration Standard Points*, respectively.
After interpolation, operator obtains the corresponding specific GM- and ER- percentage for each unknown sample. Obtaining the % GM-target and % ER-target results for each unknown sample, operator is able to simply calculate the % GM-matrix in the sample.



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

% GM Target = [(GM Target %) / (ER Target %)] x 100

E.g.: % GM target presence in unknown sample:
% GM target resulted from Real-Time PCR run: 15%
% ER target resulted from Real-Time PCR run: 75%
% GM target presence in unknown samples= (15/75)*100=20% GM target on the specific TAXA corresponding at the ER- amplified.

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. Standard Points reactions failed to amplify, but other reactions appear correct (e.g. the endo is amplified):

- Standard Points DNA was not added to the reaction wells. PCR run should be repeated.

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:	Quantitative
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 GM OLIGO Mix * (OLIGOS and Probe pre-blended mix)	1	150	2	150
MODIfinder ER OLIGO Mix * (OLIGOS and Probe pre-blended mix)	1	150	2	150
GM Standard Points	5	120	5	120
ER Standard Points	5	120	5	120
Negative Controls	2	1000	2	1000

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

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

Reaction Set-Up

Protect reagents from light exposure as far as OLIGO Mix reagents are photosensitive.
Before setting the analysis, we strongly advice to leave the reagents to warm up at room temperature. Vortex briefly GM and ER OLIGO mixes , afterwards spin to collect contents at the bottom of the vials. Spin GM and ER GENERase Mastermix before opening it.

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

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

Then transfer MODIfinder GM- WORKING Mastermix, MODIfinder ER- WORKING Mastermix, Standard Points and samples in the plate as follow*: (each unknown sample must be screened for both targets - we recommend to set-up a double standard curve for both targets -GM and ER- for a more accurate analysis).*

Reagents per well	**Volume**
Unknown Sample	12 µl
Standard Points and Negative Control	
MODIfinder WORKING Mastermix	18 µl
Final Volume	30 µl

Detector Setup

Target	**Reporter Dye**	**Quencher Dye**
GM TARGET	FAM	BHQ1-NFQ
ER TARGET	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

The analysis was carried out correctly if the following conditions are all met at the same time:

I. Negative Control should not show any significant amplification curve.

II. All the Sandard Points shows an amplification curve.

The standard curve must respect the following parameter: correlation coefficient (R^2) ≥ 0.985.

Results for unknown samples:

% GM Target = [(GM Target %) / (ER Target %)] x 100

The detection limit for each matrix can be evaluated by the user after carrying out in-house tests.

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.