eurofins | GeneScan

GMO*Quant*

GMO*Quant* (LR)

Event MON810 Corn

Test kit for event-specific quantification of MON810 corn DNA

Cat. No. 5125207801

GMO*Quant* (LR) Event MON810 Corn_ID2226 V2 13.12.2017

eurofins | GeneScan

Eurofins GeneScan Technologies GmbH
Engesser Str. 4
79108 Freiburg, Germany
Phone: + 49-(0)761-5038-100
Fax: + 49-(0)761-5038-111
kits@eurofins.com
www.eurofins.com/kits

© 2017 Eurofins GeneScan Technologies GmbH, all rights reserved.

eurofins | GeneScan

Table of Contents:

1. INTENDED USE 4

2. INTRODUCTION 4

3. PRINCIPLE OF THE TEST 5

4. MATERIALS 7

4.1 Material required but not supplied 9

5. RECOMMENDATIONS AND WORKING PRECAUTIONS 10

6. PROCEDURAL NOTES 11

7. TEST PROCEDURE 12

7.1 General Description 12

7.2 Calculation and Setup of Reactions 13

7.3 Setup and Programming of Plate Documents 15

7.4 Sample DNA 16

7.5 Plate Setup ”Quantitative Run” 17

7.6 Procedure 18

8. RESULTS 19

8.1 Evaluation 20

8.2 Interpretation of Results 22
8.2.1 Parameters and Acceptance criteria 22
8.2.2 Homogeneity / DNA Extraction 23
8.2.3 Inhibition Control with Sample Dilution 23

9. PRODUCT WARRANTIES AND SATISFACTION GUARANTEE 25

10. PRODUCT USE LIMITATIONS 26

11. TECHNICAL SUPPORT 27

Test kits, their components and instructions for use are subject to alterations. They are intended for research purposes only.

GMO*Quant* Event MON810

Kit for the event-specific quantification of DNA from MON810 corn in food and feed samples

1. INTENDED USE

The quantitative Event MON810 DNA detection kit provides materials for the event-specific quantitative measurement of MON810 corn DNA derived from food, feed, seed and environmental samples.

2. INTRODUCTION

DNA detection methods take advantage of the relative stability of DNA, which resists the processing steps used in food and feed production better than e.g. proteins. DNA can be detected and quantified with high sensitivity and accuracy with RT-PCR (real-time polymerase chain reaction).

3. PRINCIPLE OF THE TEST

The GMO*Quant* Event MON810 Corn test kit employs a quantitative real-time PCR system for ABI-PRISM® 7500 and equivalent real-time PCR cyclers with two single systems for the detection of corn (species or corn reference) and MON810 Corn (GMO).
DNA segments are amplified with two specific primers. PCR products are measured real-time with a target-specific oligonucleotide probe labelled with two fluorescent dyes: FAM as a reporter dye at its 5′-end and a non-fluorescent quencher dye (NFQ) at its 3′-end. Due to the 5′-nuclease activity of the Taq DNA polymerase the probe is cleaved, leading to increased fluorescence. The kit contains ROX as normalising dye in a concentration apt for ABI PRISM® 7500 and similar machines.[1]

The detection systems are published by the JRC.
The GMO system is event-specific.
The reference system is species specific and detects a segment of the hmgA (high mobility group A) housekeeping gene of corn.

[1] for ABI 7900, 7700, 7000 and similar machines please preferentially use our special high-ROX kits.

eurofins GeneScan

GMO*Quant* (LR)
Event MON810 Corn

Test kit for quantification of MON810 corn DNA
Cat. No. 5125207801

Two standard curves are generated, one each with the GMO system and the species reference system.
For each standard curve four calibration points are determined with the calibration DNA standards included in the kit. Subsequently the percentage of GMO DNA in the unknown DNA sample is calculated by relative quantification.

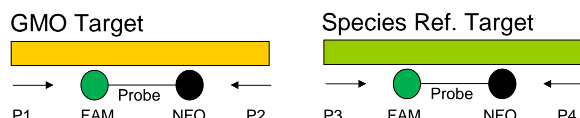

Figure 1: FAM as reporter dye and a non-fluorescent quencher are attached to the 5' and 3' ends of the probes for GMO and species reference.

4. MATERIALS

The GMOQ*uant* Event MON810 Corn kit contains the following reagents for 2x 48 reactions:

1. MasterMix (LR) GS-P-07.066 • event MON810 (RED CAPS)
   PCR reaction mix incl. primers, probe, dNTPs, hot-start polymerase, buffer; 1000 µL
2. MasterMix (LR) Corn HMGa (GREEN CAPS)
   PCR reaction mix incl. primers, probe, dNTPs, hot-start polymerase, buffer; 1000 µL
3. Calibration Standards 1-5 for corn reference and MON810: 5 x 65 µL of calibration DNA
   (see also table 1)

   Use #1-4 for corn reference
   (#1: WHITE CAP, #2-4 STRIPED VIOLET CAPS)

   Use #2-5 for MON810
   (#2-4 STRIPED VIOLET CAPS, #5: VIOLET CAP)
4. 1.0% MON810 Corn DNA (CLEAR CAP)
   65 µL quantification control,
   made from cornmeal containing:
   1% MON810 (w/w), ERM BF413d (HGE 0.57%).

Store all reagents light protected at –20°C.

MATERIAL	Copy numbers/rxn Corn Reference	Copy numbers/rxn MON810
Standard 1	81,920	
Standard 2	10,240	10,240
Standard 3	1,280	1,280
Standard 4	160	160
Standard 5		40

Table 1: Shown are the assigned target copy numbers and DNA amounts per 5 µL of the calibration standards, which are made of plasmids.

4.1 Material required but not supplied

- Optical tubes or plates and optical caps or seals
- Accuracy pipette to deliver 5 µL and 20 µL
- Vortex mixer
- Centrifuge (1500 x g, preferably refrigerated)
- Centrifuge for micro titer plates (preferably refrigerated)
- ABI PRISM® 7500 / 7500 Fast or Agilent MX3005P or MX3000 instrument or equivalent RT-PCR instrument

5. RECOMMENDATIONS AND WORKING PRECAUTIONS

For DNA extraction use only molecular-biology grade reagents and sterile-filtrated, deionised water (DNA-free) and 0.1x TE buffer for DNA dilutions (Tris-HCl pH=8.0 c=1 mmol/L; EDTA pH=8.0 c=0.1mmol/L):

Maintain separate working areas for DNA preparation, reaction setup, amplifications and reaction product analyses. Never transfer amplified products into the reaction setup, or extraction area.

Use filter-plugged pipette tips in order to avoid possible cross-contamination. Use only powder-free gloves and change them frequently during the process.

Clean lab-benches and equipment periodically with 10% bleach solution. To obtain reliable quantification results all pipettes have to be calibrated regularly.

6. PROCEDURAL NOTES

A thorough understanding of this package insert is necessary for successful use of the product. Reliable results will only be obtained when precise laboratory techniques are employed and the package insert is accurately followed.

The standard curve must be included in every quantification run.

Do not mix kit components of various kit lots within one run. Do not use any component beyond the expiration date of the kit.

After removing reagents from the refrigerator, allow them to thaw slowly and mix them by vortexing and briefly centrifuge before use. Keep the MasterMix cooled during the working steps.

Thaw only the MasterMix volumes needed for the run. Unused reagents should be stored at –20°C. Standards and positive controls should be mixed by vortexing and briefly centrifuged before use.

7. TEST PROCEDURE

7.1 General Description

Different assay formats are possible: For one laboratory sample either one DNA extraction with two or more PCR-replicates or two parallel (“A” and “B”) DNA-extractions (as recommended by certain national and international standards) may be analysed. Different numbers of replicates per DNA sample (e.g. triplicates in case of only one DNA-sample) may be chosen. The mean value of several replicates will yield increased accuracy of the analysis. Furthermore, different ways to check sample DNAs for inhibitory effects may be applied – e.g. analysing the DNA in two defined dilutions. This can either be done in a preceding monitor run or, according to the user´s preference, it can also be done in the quantification run without prior monitor run.
The proceedings described are made for the following format and assumption:

DNA Test Samples
Two independent DNA extracts yielding an “A” and a “B” sample DNA are taken. Each DNA is analysed in two different defined concentrations (dilution 1 (dil.1) and dilution 2 (dil.2)). For example, each DNA could be analysed undiluted (dil. 1) and in a known dilution, e.g. a fivefold dilution with water or TE (dil. 2). Each DNA dilution is analysed with a single determination (no replicates).

Calibration curves
Four concentrations per target are analysed in duplicate each.

7.2 Calculation and Setup of Reactions

Calculate the number of reactions and amount of equivalent MasterMix volume before thawing the reagents and starting the practical work. The calibration standard and positive control materials are supplied with the kit.

eurofins | GeneScan

GMO*Quant* (LR)
Event MON810 Corn

Test kit for quantification of MON810 corn DNA
Cat. No. 5125207801

The MasterMix solutions supplied are pre-made reagents ready for direct use. Quantitative analyses require at least the following reactions:

Reaction Type		Sample no per run 1: MON810 Rxns	Sample no per run 1: Corn Rxns	Sample no per run 9: MON810 Rxns	Sample no per run 9: Corn Rxns
Sample DNA Two Extracts in 2 Dilutions	DNA #1A (dil.1)	**1**	**1**	**9**	**9**
	DNA #1A (dil.2)	**1**	**1**	**9**	**9**
	DNA #1B (dil.1)	**1**	**1**	**9**	**9**
	DNA #1B (dil.2)	**1**	**1**	**9**	**9**
DNA Standards 4 Calibration Points in Duplicates	MON810 STND	**8**	**-**	**8**	**-**
	Corn Ref STND	**-**	**8**	**-**	**8**
Controls	No Template Control (NTC)	**2**	**2**	**2**	**2**
	Quantification Control	**2**	**2**	**2**	**2**
	Number of Rxns	**16**	**16**	**48**	**48**

Table 2: Number of reactions needed for 1 or 9 samples.

For setup and evaluation information for your individual cycler, please send an email to kits@eurofins.com or see our website www.eurofins.com/kits.

7.3 Setup and Programming of Plate Documents

Before starting the practical work, program the plate document. For general and more detailed instructions please refer to the user guide of the instrument and respective software version.

Program the ‘Thermocycler Conditions‘ as given below. Before amplification cycling the polymerase activity must be activated by thermal incubation at 95°C for 10 min.

The PCR temperature profile is:

Temperature	Time	
95°C	10 min	
95°C	15 sec	45 cycles
60°C	90 sec	45 cycles

Table 3: Temperature profile

7.4 Sample DNA

Absolute DNA amount and purity (meaning: free of PCR inhibiting substances) affect overall sensitivity as well as accuracy of the analysis. Low DNA amount subjected to analysis results in poor LOD and LOQ with regard to the specific test sample. Insufficiently pure DNA displaying inhibitory effects increase inaccuracy or even lead to totally biased results.

It is recommended to use 200 ng DNA of the species of interest per reaction. The concentration of DNA should be adjusted accordingly prior to the setup of the reactions. DNA amount can be determined prior to analysis by one of the following methods:
- Real Time PCR monitor run (preferred method)
- Fluorimetric or spectrophotometric or other physical measurements, e.g. Gel electrophoresis may be applied.

Especially the spectrophotometric methods can suffer significant errors due to interfering DNA impurities or partial DNA-denaturation/degradation. When analysing samples for which there is a lot of experience in the extraction yield and purity, DNA measurement may be omitted.

7.5 Plate Setup "Quantitative Run"

The following plate document (table 4) shows the distribution of reactions used for quantitative analysis of 9 species samples.

	1	2	3	4	5	6	7	8	9	10	11	12
A	St1	St1	St2	St2	St3	St3	St4	St4	NTC	NTC	NTC	NTC
B	St2	St2	St3	St3	St4	St4	St5	St5	1%	1%	1%	1%
C	1a	1b	1a	1b	1a D	1b D	1a D	1b D	2a	2b	2a	2b
D	2a D	2b D	2a D	2b D	3a	3b	3a	3b	3a D	3b D	3a D	3b D
E	4a	4b	4a	4b	4a D	4b D	4a D	4b D	5a	5b	5a	5b
F	5a D	5b D	5a D	5b D	6a	6b	6a	6b	6a D	6b D	6a D	6b D
G	7a	7b	7a	7b	7a D	7b D	7a D	7b D	8a	8b	8a	8b
H	8a D	8b D	8a D	8b D	9a	9b	9a	9b	9a D	9b D	9a D	9b D

Table 4: Plate layout for 9 samples: green: Species ref. MasterMix, orange: GM MasterMix; NTC = no template control, St = Standard (row A: Species ref. standards, row B: GM standards); a and b = duplicates of sample; D = Dilution of sample

In this example, 48 reactions are needed for each system. Thirty-six of these reactions in each system will analyse 9 different test samples (#01 to #09), where DNA-extracts a and b are tested at two dilutions (e.g. undil. and dil. fivefold).

eurofins GeneScan

GMO*Quant* (LR)
Event MON810 Corn

Test kit for quantification of MON810 corn DNA
Cat. No. 5125207801

The remaining 12 reactions in each system will analyse the 4 calibration standards (Stnd1 to Stnd4 for corn species reference, Stnd 2 – Stnd 5 for MON810) in duplicate, two no template controls in duplicate and two quantification controls (1% MON810).

7.6 Procedure

Before starting the practical working steps switch on the instrument and make sure the plate document is properly set and programmed.
Allow the MasterMix reagents to thaw and mix them by vortexing. Centrifuge carefully before use.

1. Add 20 µL of the MasterMix into appropriate tubes or wells of the plate according to the programmed plate document.
2. Add 5 µL of DNA solution (Standards, Controls or Unknowns) to the inner side of the tube or well.
3. Carefully close all tubes/plates with optical caps/seals.
4. Centrifuge tubes or plate carefully at low speed.
5. Place tubes or plate into the thermal block of the instrument, close lid and start the run.

8. RESULTS

The four calibration standards analysed in both systems (GM corn and corn reference) allow for the calculation of the relative number of copies for both targets in every sample. The number of copies of GM corn is normalised for the amount of corn genomic DNA in each sample by dividing it by the number of copies of corn reference in the same volume, of the same dilution, of the same sample. Then, the ratios of GM corn/corn reference can be compared among all samples analysed. A sample of known GMO concentration (DNA from 1.0% MON810 corn certified reference material) is included in the analysis, this sample serves as a control of the calibration standards and the quantification process and should not show a deviation of more than 25% from the expected result.
Quantification of the relative number of copies of each target DNA can be done with the standard curve function within the ABI 7500 SDS software. However, our recommendation is to export the Ct values from the SDS software to a local spreadsheet (e.g. Microsoft Excel™), with subsequent standard curve calculations made by the user, because this saves individual programming of plates (Slightly different results may be obtained with both ways due to rounding errors).

The following section describes a short protocol for data analysis of quantitative runs.

8.1 Evaluation

Refer to your cycler´s manual for details. An evaluation (Excel™) sheet can be requested at kits@eurofins.com.

Threshold: The threshold should be placed in the region of exponential amplification across all of the amplification plots. This region is depicted in the log view of the amplification plots as the portion of the plot, which is linear. The threshold line should neither be placed in the plateau phase nor in the initial linear phase of amplification. Thresholds are typically in the range 0.03 - 0.1.

Baseline:
ABI 7500/7500 Fast: Set manually, 3-15
Agilent Mx3000P™/3005P™: Adaptive

The percentage of MON810 corn in each sample is calculated as the ratio of the MON810 corn quantity divided by the corn reference quantity. The spreadsheet table can have implemented functions to calculate the MON810 corn DNA content in percent.

Sample Name	MON810 Quantity	Corn Ref. Quantity	Rel. MON810 DNA Content	Result MON810 (% HGE)
1/A dil.1	270	50320	0.54%	0.59% +/-0.05%
1/B dil.1	278	49850	0.56%	
1/A dil.2	62	9958	0.62%	
1/B dil.2	59	9211	0.64%	

This example (table 5) shows a possible relative Roundup Ready™ MON810 corn DNA content, calculated using the procedure above. Two DNA extracts were prepared in parallel and two dilutions of these DNA extracts were analysed. The mean MON810 corn DNA content as well as the corresponding standard deviation is calculated from the four individual results obtained with the DNA sample dilutions.

The actual unit of measurement is "percent HGE (haploid genome equivalents) GMO", because the calibration standards are made of plasmid DNA with 1:1 ratio GMO/species. Thus a heterozygous material of a 1% GMO:non-GMO mixture on a weight/weight basis should result in a measured % HGE GMO value of approximately 0.5% (the certified reference material value is 0.57%).

In kernels and derived products, maternal effects can result in small deviations from this value, as the triploid endosperm is of two third maternal origins.

8.2 Interpretation of Results

Depending on the assay format chosen, the interpre-tation of the results may differ. Here it is described for the recommended format.

8.2.1 Parameters and Acceptance criteria

These criteria have to be fulfilled:

- Correlation coefficient of regression line (R^2) ≥ 0.98
- The average value of the slope of the standard curves should be approximately in the range of - 3.1 ≥ slope ≥ - 3.6, corresponding to amplification efficiencies of 90% to 110%. (*Efficiency [%] = [$10^{(-1/slope)}$] -1) x 100*)
- Deviation of quantification result of quantification control (prepared from certified reference material - CRM) from expected result +/- 30%
- No template controls (NTC) show no amplification

8.2.2 Homogeneity / DNA Extraction

If independently extracted DNAs show significant deviations between "A" and "B", this may be due to sample inhomogeneity (if differences in the quantification results) or to non-uniformities in the DNA-extraction efficiency (if differences in the copy numbers).
If the two quantification results and/or DNA copy numbers for the species show significant differences, repeat DNA extraction and homogenize sample more thoroughly.

8.2.3 Inhibition Control with Sample Dilution

At least two dilutions of the DNA test sample should be used to detect inhibitory effects.
As inhibition control the experimentally determined dilution factors in the species specific as well as in the GMO specific systems are calculated using the following equation:

$$Dilution\ factor = \frac{Copy\ number_{undiluted\ sample}}{Copy\ number_{diluted\ sample}}$$

The calculated dilution factor is than compared with the dilution of the sample.

Deviation of +/- 20% can be accepted.
Deviation from calculated dilution factor to actual dilution factor:

Dilution of sample	Acceptable calculated dilution factor
1:5	1:4 to 1:6
1:10	1:8 to 1:12
1:20	1:16 to 1:24

If deviations occur, samples can be accepted after case to case evaluation. But deviation of quantification of duplicates should not be greater than -50% to +100%. For greater deviations, the analysis has to be repeated.

9. PRODUCT WARRANTIES AND SATISFACTION GUARANTEE

Eurofins GeneScan Technologies GmbH (“GeneScan”) warrants the products manufactured by it will be free of defects in materials and workmanship when used in accordance with the applicable instructions for a period equal to or shorter than one year from the date of shipment of the product(s) or the expiration date marked on the product packaging under the storage conditions, recommended in the instructions and/or on the package. Application protocols published by GeneScan are intended to be only guidelines for the buyers of the products. Each buyer is expected to validate the applicability of each application protocol to his individual application. GeneScan makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose.

GeneScan’s sole obligation with the respect to the foregoing warranties shall be, at its option, to either replace or to refund the purchase price of the product(s) or part thereof that proves defective in materials or workmanship within the warranty period, provided the customer notifies GeneScan promptly of any such defect.

eurofins GeneScan
GMO*Quant* (LR)
Event MON810 Corn
Test kit for quantification of MON810 corn DNA
Cat. No. 5125207801

GeneScan shall not be liable for any direct, indirect or consequential damages resulting from economic loss or property damages sustained by buyer or any customer from the use of the product(s). A copy of Eurofins GeneScan Technologies GmbH terms and conditions can be obtained on request, and is also provided in our price lists.

10. PRODUCT USE LIMITATIONS

The GeneScan Technologies GMO*Quant* Kit is developed, designed, and sold for research purposes only. It is not to be used for diagnostic purposes or analysis of food and feed unless expressly cleared for that purpose by the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of many of the materials described in this text.

11. TECHNICAL SUPPORT

If you have any questions or experience any difficulties regarding this kit or Eurofins GeneScan Technologies products in general, please do not hesitate to contact us. Our customers are a major source of information regarding advanced or specialised uses of our products. This information is helpful to other scientists as well as to the researchers at GeneScan Technologies.

We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques. For technical assistance and more information please call Eurofins GeneScan Technologies or your local distributor.