GMO*Screen* RT
35S/NOS/FMV IPC

Test kit for qualitative RT-PCR detection of the 35S and FMV promoter and the nos terminator on

- **Applied Biosystems® 7500/Fast**

Cat. No: 5421220301 (LR)

- **Roche LightCycler® 480 Instrument**
- **Agilent Mx3000P/Mx3005P™**
- **Agilent AriaMx™**
- **Bio-Rad CFX96 Touch™**

Cat. No: 5421220302 (NR)

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10 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 also a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at Eurofins GeneScan.
We therefore encourage you to contact us if you have any suggestions regarding product performance or new applications and techniques.

For technical assistance and more information please call Eurofins GeneScan Technologies or your local distributor.

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GMO*Screen* RT
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Test kit for real-time PCR detection of CaMV 35S and FMV promoter and nos terminator
Cat. Nos: 5421220301 and 5421220302

Table of Contents:

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

GMO*Screen* RT 35S/NOS/FMV IPC Kit

Kit for real-time multiplex PCR detection of the CaMV 35S and FMV promoter and the nos terminator

1 INTENDED USE

The real-time 35S/NOS/FMV IPC DNA detection kit provides materials for the qualitative measurement of three regulatory DNA sequences often used in GMO production, the CaMV 35s promoter of the Cauliflower Mosaic Virus, the FMV 34s promoter of the Figwort Mosaic Virus and the nos terminator from the nopalin synthetase gene of *Agrobacterium tumefaciens*. Detection of one or more of these sequences strongly indicates the presence of a genetic modification in the sample DNA.

The GMO*Screen* RT 35S/NOS/FMV IPC kit is a highly economical tool for a fast and efficient GMO screening of food and feed. Because a positive control reaction is included for every sample, false-negative results caused by PCR inhibition can be excluded.

The GMO*Screen* RT 35S/NOS/FMV IPC test kit employs a real-time PCR system validated for either Applied Biosystems® 7500/Fast or Agilent Mx3005P/Mx3000P™, Roche LightCycler® 480, Agilent AriaMx™ or Bio-Rad CFX96 Touch™.
The kit cannot be used on other RT-PCR cyclers without prior thorough validation.

The kit is part of GeneScan's comprehensive product line for screening, identification and quantification of genetically modified organisms, called GMO*Screen*, GMO*Ident*, and GMO*Quant*.

2 PRINCIPLE OF THE TEST

The GMO systems are element specific and detect sequences of the CaMV 35s promoter, the FMV 34s promoter and the *Agrobacterium* nos terminator.

The 35S detection system in the kit exhibits an enlarged specificity/sensitivity as compared to similar 35S detection systems, e.g. the ISO 21570 system.

An internal positive control (IPC) is contained in the MasterMix which is amplified in parallel and indicates inhibition, if it occurs.

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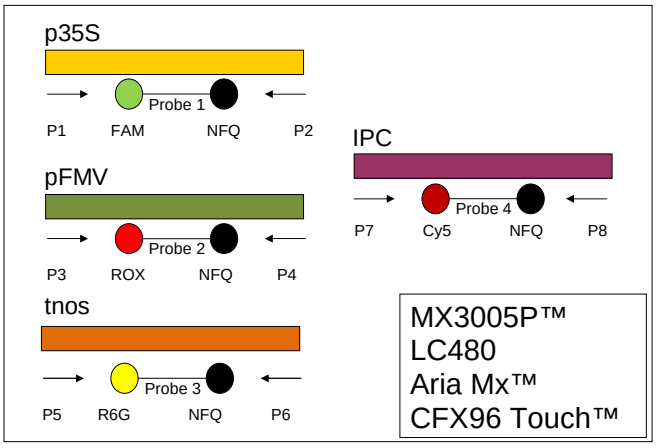
DNA segments are amplified with specific primers. PCR products are measured during each cycle (real-time) with target-specific TaqMan™ oligonucleotide probes labeled with fluorescent reporter dyes at their 5′-end.

The kit for Mx3005P/Mx3000P, AriaMx™ , CFX96 Touch™ and LC480 uses the following dyes: FAM™, R6G, ROX™ and Cy5® for the IPC.

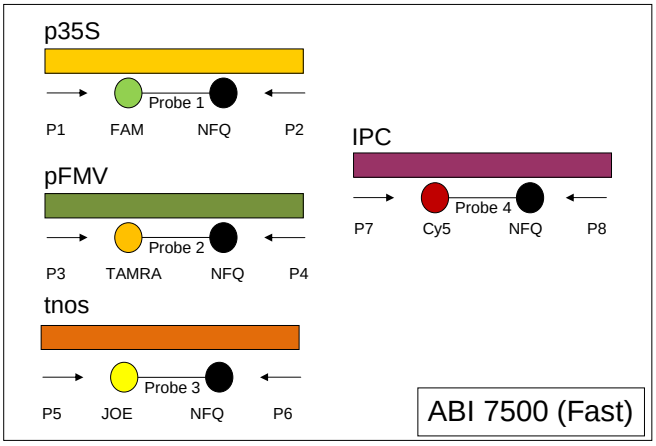
The kit for ABI Fast 7500 uses:
FAM™, JOE™, TAMRA™ and Cy5® for the IPC.
Furthermore, the kit contains ROX™ as normalizing dye.

Non-fluorescent quenchers (NFQ) are used as quencher dyes.
Due to the 5′-nuclease activity of the Taq DNA polymerase, the probe is cleaved, leading to increased fluorescence.
The fluorescence is measured and this leads to amplification curves.

Taking two criteria, threshold cycles and fluorescence, into account, the final evaluation is done.



Kit for MX3005P™, LC480, AriaMx™ and CFX96 Touch™ cat # 5421220302: FAM™, ROX™, R6G and Cy5® are the fluorescent reporter dyes attached to the 5' ends of the probes for GMO screening and for the IPC (internal positive control). Non-fluorescent quenchers (NFQ) are used for quenching.



Kit for ABI 7500 (Fast), cat # 5421220301: FAM™, TAMRA™, JOE™ and Cy5® are the fluorescent reporter dyes attached to the 5' ends of the probes for GMO screening and for the IPC (internal positive control). Non-fluorescent quenchers (NFQ) are used for quenching.

2.1 List of GMOs detected

The following list shows selected GMO plants which contain the 35S promoter/nos terminator/FMV promoter and are detected with this kit:

OECD Identifier / Species	Event	FMV	35S	nos
Soybean				
MON-Ø4Ø32-6	GTS 40-3-2		+	+
MON-89788-1	MON89788	+		
ACS-GMØØ5-3	A2704-12		+	
ACS-GMØØ6-4	A5547-127		+	
DP-356Ø43-5	356043		+	
MON-877Ø5-6	MON87705	+		
MST-FGØ72-3	FG72			+
SYN- ØØØH2-5	SYHT0H2	+	+	+
MON-87712-4	MON87712		+	
DD-Ø26ØØ5-3	G94-19, G-168, G94-6, G94-1		+	+
ACS-GMØØ4-2	A2704-21		+	
ACS-GMØØ2-9, ACS-GMØØ1-8	W98, W62		+	+
ACS-GMØØ3-1	Event GU262		+	
ACS-GMØØ8-6	A5547-35		+	+
Corn				
SYN-BTØ11-1	Bt11		+	+
MON-ØØØ21-9	GA21			+
DAS-Ø15Ø7-1	TC1507		+	

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OECD Identifier / Species	Event	FMV	35S	nos
Corn (continued)				
DAS-59122-7	DAS-59122-7		+	
ACS-ZMØØ3-2	T25		+	
SYN-IR6Ø4-5	MIR604			+
MON-ØØ81Ø-6	MON810		+	
MON-88Ø17-3	MON88017		+	+
MON-89Ø34-3	MON89034	+	+	+
MON-ØØ6Ø3-6	NK603		+	+
SYN-IR162-4	MIR162			+
MON-8746Ø-4	MON87460		+	+
MON-87427-7	MON87427		+	+
SYN-E3272-5	3272			+
SYN-Ø53Ø7-1	Event 5307			+
DP-Ø9814Ø-6	Event 98140		+	
MON-87411-9	MON87411		+	
MON-874Ø3-1	MON87403		+	
DP-ØØ4114-3	DP-004114-3		+	
SYN-ØØØJG-2	MZHG0JG	+	+	+
SYN-EV176-9	Bt 176		+	
MON-ØØ863-5	MON863		+	+
DKB-89614-9	DBT418		+	
ACS-ZMØØ4-3	CBH-351		+	+
n/a	Bt10		+	+
DAS-Ø6275-8	TC 6275		+	
ACS-ZMØØ2-1	T14		+	
DP-32138-1	DP-32138-1		+	
SYN-ØØØ98-3	MZIR098		+	+

OECD Identifier / Species	Event	FMV	35S	nos
Cotton				
MON-ØØ531-6, MON-ØØ757-7, MON-89924-2	1076, 757, MON531		+	+
MON-15985-7	MON15985		+	+
ACS-GHØØ1-3	LLCotton25		+	+
MON-Ø1445-2	MON1445	+	+	+
MON-88913-8	MON88913	+	+	
BCS-GHØØ4-7	T304-40		+	+
BCS-GHØØ5-8	GHB119		+	+
MON-887Ø1-3	MON88701		+	+
SYN-IR1Ø2-7	COT102			+
BXN-1Ø211-9, BXN-1Ø215-4, BXN-1Ø222-2	10224, 10222, 10215, 10211		+	
n/a	42317, 31803, 31707, 31808, 31807		+	
SYN-IR67B-1	COT67B			+
JK-Bt Event-1	Event-1 (JKC 738 Bt-a)		+	+
Rice				
ACS-OSØØ1-4, ACS-OSØØ2-5	LLRICE62, LLRICE06		+	
BCS-OSØØ3-7	LLRice601		+	
n/a	LLRice604		+	+
n/a	TT51-1 (BT63)			+
n/a	Event 166		+	+
n/a	Kefeng8			+

OECD Identifier / Species	Event	FMV	35S	nos
Sugarbeet				
KM-ØØØH71-4	H7-1	+		
SY-GTSB77-8	GTSB 77 (T9100152)	+	+	
ACS-BVØØ1-3	Event T120-7		+	
Canola				
MON-ØØØ73-7	RT73 (= GT73)	+		
ACS-BNØØ5-8	MS8 (DBN230-0028)			+
ACS-BNØØ3-6	RF3 (DBN212-0005)			+
ACS-BNØØ8-2	T45		+	
MON-883Ø2-9	MON88302	+		
ACS-BNØØ4-7	MS1 (B91-4)			+
ACS-BNØØ1-4	RF1 (B93-101)			+
ACS-BNØØ2-5	RF2			+
ACS-BNØØ7-1	Topas 19/2		+	
CGN-89111-8, CGN-89465-2	23-18-17, 23-198		+	
ACS-BNØ11-5	Westar Oxy-235		+	+
ACS-BNØØ9-3	Liberator C/6Ac		+	
ACS-BNØ1Ø-4	Falcon GS 40/90		+	
MON-89249-2	GT200 (= RT200)	+		

This list is based on different publicly available sources, it is subject to changes. Eurofins GeneScan assumes no liability for the completeness and accuracy of the list.

3 MATERIALS

The GMO*Screen* RT 35S/NOS/FMV IPC (LR) kit contains the following reagents for 96 reactions:

2x Qualitative real-time MasterMix GS-P-09.136 (IPC) for 35S/NOS/FMV detection, each composed of

- 650 µL BasicMix BM (NR) GS-P-26.012 • EFG*i* TP 2x (NR) 1.5U
- 390 µL OligoMix OM QL RT IPC (LR or NR) GS-P-09.136 35S/NOS/FMV

Mix prior to use in empty vial (labels included)

2x positive control DNA
Plasmid DNA pGSE-P-09.136
10 copies/µL, 50 µL

1x 150 µL DNA stabilization buffer (for NTCs)

Store all reagents light protected at –20°C.

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3.1 MATERIALS NOT INCLUDED

- Optical plates and optical seals
- Accuracy pipettes
- Vortex mixer
- Centrifuge (1500 x g)
- Centrifuge for micro titre plates
- Applied Biosystems® 7500 Fast, Agilent Mx3005P™ (with appropriate filters for the dyes used in this kit), Roche LightCycler® 480 instrument I/ II, Agilent AriaMx™ (with appropriate filters for the dyes used in this kit) or Bio-Rad CFX96 Touch™

On LC480, Color Compensation must be performed before the first test is run. Please use our color compensation kit with cat. no. 5427200401.

4 RECOMMENDATIONS AND WORKING PRECAUTIONS

For DNA extraction, use only molecular-biology grade reagents and sterile-filtrated, deionized 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=0.1 mmol/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 results, all pipettes have to be calibrated regularly.

5 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.
Do not mix kit components of various kit lots. 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. Briefly centrifuge before use. Thaw and prepare only as much MasterMix from the components as needed for PCR.
Keep the MasterMix cooled during the working steps.
The composed MasterMix can be stored up to 4 hours in the refrigerator, but must not be used after this period.
Unused reagents should be stored at -20°C. MasterMix and positive controls should be mixed by vortexing and briefly centrifuged before use.

6 TEST PROCEDURE

6.1 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 analyzed. Different numbers of replicates analyzed per DNA sample (e.g. triplicates in case of only one DNA sample) may be chosen.
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 extract is analyzed with a single determination (no replicates).

Controls
The number of positive and negative controls given in the setup-description is compulsory and indispensable for evaluation of the results.

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6.2 Sample DNA

Absolute DNA amount and purity (meaning: free of PCR inhibiting substances) affect overall sensitivity of the analysis. Low DNA amount subjected to analysis results in poor LOD with regard to the specific test sample. Insufficiently pure DNA displaying inhibitory effects may even make testing impossible.

It is recommended to use the sample DNA undiluted for PCR or to use a minimum of 250 ng DNA per reaction. In case inhibitors are present in the DNA, dilution of the sample DNA is feasible. However, it needs to be considered that the practical/sample LOD for the individual analysis of the sample will be affected and – in case the DNA amount used is too small – may not reach the method LOD, which means that the sensitivity for the test is decreased.

The concentration of DNA should be adjusted accordingly prior to the setup of the reactions. The DNA amount can be determined prior to analysis by one of the following methods:
- Real-time PCR monitor run
- Fluorimetric or spectrophotometric or other physical measurements, e.g. gel electrophoresis may be applied.

Especially the spectrophotometric methods can suffer from significant errors due to interfering DNA impurities or partial DNA denaturation/degradation.
With matrices for which extraction yield and purity are known, DNA measurement may be omitted.

It is recommended to perform an extraction control for each set of samples extracted simultaneously, i.e. a complete DNA extraction without sample material, which should subsequently undergo PCR analysis.

6.3 Calculation and Setup of Reactions

Calculate the number of reactions and amount of equivalent MasterMix volume before thawing and mixing the reagents and starting the practical work.
The MasterMix is made of pre-made reagents ready for direct use after mixing of the two components.
Add approx. 5% of volume as pipetting error compensation or use complete tubes.

Samples and extraction controls	1	8	23	46
NTCs	2	2	2	2
Pos. controls	2	2	2	2
Samples/extraction controls (duplicates)	2	16	46	92
Total no. reactions	**6**	**20**	**50**	**96**
Total MM volume	**120 µL**	**400 µL**	**1 mL**	**2 mL**
BasicMix	75 µL	250 µL	625 µL or 1 tube	1250 µL or 2 tubes
OligoMix	45 µL	150 µL	375 µL or 1 tube	750 µL or 2 tubes

Number of reactions and volumes of reagents needed for 1, 8, 23 or 46 samples (incl. extraction controls).

6.3.1 Plate Setup

The following plate document (table 4) shows a possible distribution of reactions:

	1	2	3	4	5	6	7	8	9	10	11	12
A	NTC	NTC	C+	C+	1a	1b	2a	2b	3a	3b	4a	4b
B	5a	5b	6a	6b	7a	7b	8a	8b	9a	9b	10a	10b
C	11a	11b	12a	12b	13a	13b	14a	14b	15a	15b	16a	16b
D	17a	17b	18a	18b	19a	19b	20a	20b	21a	21b	22a	22b
E	23a	23b	24a	24b	25a	25b	26a	26b	27a	27b	28a	28b
F	29a	29b	30a	30b	31a	31b	32a	32b	33a	33b	34a	34b
G	35a	35b	36a	36b	37a	37b	38a	38b	39a	39b	40a	40b
H	41a	41b	42a	42b	43a	43b	44a	44b	45a	45b	ECa	ECb

Plate layout for 45 samples + extraction control (EC); NTC = no template control; C+ = positive control; a and b = sample/DNA extraction duplicates

Every run requires two no template controls (NTC) and two positive controls (C+). It is highly recommended to test the samples in duplicate.

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6.4 Programming of the cycler

Before starting the practical work, program the plate document and the cycling conditions.

For description of the instrument programming please send an email to kits@eurofins.com and refer to the user manual of the respective instrument and software version.

6.4.1 Plate template

If you wish to use the Eurofins GeneScan evaluation sheet with automated data import, please use the detector/assay names given below.
Program the plate template with the following settings:

ABI 7500/7500 Fast

System	Detector	Reporter	Quencher
35S	9136a	FAM™	NONE
NOS	9136b	JOE™	NONE
FMV	9136c	TAMRA	NONE
IPC	9136i	Cy5®	NONE
Passive Reference:		ROX	

Agilent Mx3005P/MX3000P™

System	Assay	Reporter	pmt
35S	9136a	FAM™	1
NOS	9136b	HEX™/JOE™/VIC™	1
FMV	9136c	Texas Red®/ROX™	1
IPC	9136i	Cy5®	1

A reference dye is not used.
The fluorescence signals are scanned in the following order: (1) ROX, (2) FAM, (3) HEX, (4) Cy5.

Set filter set gain settings for all filters to pmt = 1.

Roche LightCycler®480 Instrument
Color Compensation for FAM, R6G, ROX and Cy5 is performed according to the manual.

System	Filter
35S	FAM (483-533)
NOS	VIC/HEX/Yellow555 (523-568)
FMV	Red 610 (558-610)
IPC	Cy5® (615-670)

Ramp rate is set to 4.4 for heating and 2.2 for cooling.
A reference dye is not used.

Agilent AriaMx™

System	Targets	Dyes
35S	9136a	FAM™
NOS	9136b	HEX™
FMV	9136c	ROX™
IPC	9136i	Cy5®

A reference dye is not used.

Bio-Rad CFX96 Touch™

System	Targets	Dyes
35S	9136a	FAM™
NOS	9136b	VIC™
FMV	9136c	ROX™
IPC	9136i	Cy5®

A reference dye is not used.

Cycling Conditions

The run parameters are to be programmed as follows:

Thermal Cycler Times and Temperatures		
1 HOLD	*CYCLE (45 repeats)*	
enzyme activation	*denaturation*	*annealing & extension*
10 min at 95°C	*15 sec at 95°C*	*90 sec at 60°C*
no data	*no data*	*data collection*

ABI 7500 Fast
PCR is performed in “Standard 7500” run mode.

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6.5 Procedure

Before starting the practical working steps, switch on the computer, 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. Prepare the final MasterMix by mixing the BasicMix and the OligoMix as shown above (12.5 µL of BasicMix and 7.5 µL of OligoMix per reaction).
2. Vortex and centrifuge the MasterMix.
3. Add 20 µL of the MasterMix to wells of the plate according to the programmed plate document.
4. Add 5 µL of DNA solutions (positive controls, stabilization buffer for NTCs, or sample DNA, respectively) to the inner side of the wells.
5. Carefully close the plate with the optical seal.
6. Centrifuge plate carefully at low speed.
7. Place plate into the thermal block of the instrument and start the run.

7 RESULTS

7.1 Evaluation

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

Threshold:

ABI 7500/7500 Fast and Agilent Mx 3005/3000P:
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.

Agilent AriaMx™:
Auto calculated threshold with default background based threshold settings can be used:
Cycle range: 5 thru 9
Sigma multiplier: 10

Bio-Rad CFX96 Touch™:
Auto calculated threshold can be used but should be checked visually.

Baseline:

ABI 7500/7500 (Fast): Set manually, 3-15 or auto baseline settings
Agilent Mx3005/3000P™: Adaptive

Roche LightCycler® 480:
Choose "Abs Quant/2nd Derivative Max" and High Sensitivity settings to obtain CP values.
Choose "Endpoint Genotyping" analysis mode to obtain Endpoint Fluorescence.

Agilent AriaMx™:
Auto baseline correction can be used to adjust the Baseline Cycle Range for each displayed target.

Bio-Rad CFX96 Touch™:
Baseline Subtracted Curve Fit and Fluorescence Drift Correction should be applied.
Auto calculated

7.2 Interpretation of Results

Export Ct values to the Excel™ sheet provided (please request to kits@eurofins.com), or create an own evaluation sheet following the parameters below, where you will find an overview of the algorithms applied for scoring of the results in our Excel™ Evaluation Sheet.

Definitions

Threshold cycle (Ct/Cp/Cq) Cut-offs	35S Cut-off-1:	Mean Ct (C+)	-10
	FMV Cut-off-1:	Mean Ct (C+)	-10
	nos Cut-off-1:	Mean Ct (C+)	-10
	IPC Cut-off-1:	Mean Ct (NTC)	-3
	35S Cut-off-2:	Mean Ct (C+)	+8
	FMV Cut-off-2:	Mean Ct (C+)	+9
	nos Cut-off-2:	Mean Ct (C+)	+8
	IPC Cut-off-2:	Mean Ct (NTC)	+3
Endpoint fluorescence (EPF)- Limits[1]	35S:	Mean EPF (C+)	x 0.2
	FMV:	Mean EPF (C+)	x 0.2
	nos:	Mean EPF (C+)	x 0.2
	IPC:	no EPF-Limit	
Outliers	Maximum acceptable outliers (C+): 0 Maximum acceptable outliers (NTC): 0		

[1] Depending on your cycler the final intensity value , called Endpoint fluorescence (EPF) is termed as: ΔR, dR, Rn, RFU, or EF

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Inhibition control, scoring of IPC

Threshold cycle (Ct/Cp/Cq) = Ct	Results
Ct_{IPC} Cut-off-1 ≤ Ct_{IPC} sample ≤ Ct_{IPC} Cut-off-2	Sample valid
Ct_{IPC} sample < Ct_{IPC} Cut-off-1	Check amplification!
Ct_{IPC} sample > Ct_{IPC} Cut-off-2	Sample inhibited
No Ct_{IPC}	Sample inhibited
***Note*:** It is generally recommended to check the sample amplification curves for sigmoid amplification. In case of a Ct/ Cp/ Cq value of 40.0 or "Check amplification!" th linear scale amplification plot must be checked critically for presence or absence of a sigmoid PCR amplification signal. If a sigmoid curve is observed, the sample is valid; If not, the sample is inhibited.	

Test reaction GMO, scoring of 35S, FMV or nos

Threshold cycle (Ct/Cp/Cq) =Ct	Endpoint fluorescence dRN/dR/EF= EF	Result
Ct_{GMO} Cut-off-1 ≤ Ct_{GMO} sample ≤ Ct_{GMO} Cut-off-2	EF_{GMO} sample ≥ EF_{GMO} Limit	Reaction positive
Ct_{GMO} sample < Ct_{GMO} Cut-off-2	EF_{GMO} sample < EF_{GMO} Limit	Check amplification!
Ct_{GMO} sample < Ct_{GMO} Cut-off-1	EF_{GMO} sample ≥ EF_{GMO} Limit	Check amplification!
Ct_{GMO} sample > Ct_{GMO} Cut-off-2	EF_{GMO} sample ≥ EF_{GMO} Limit	Reaction positive
Ct_{GMO} sample > Ct_{GMO} Cut-off-2	EF_{GMO} sample < EF_{GMO} Limit	Reaction negative
No Ct_{GMO}	-	Reaction negative
***Note*:** In case of a Ct value of 40.0 or "Check amplification!" the linear scale amplification plots must be carefully checked for presence or absence of a sigmoid PCR amplification signal. If a sigmoid curve can be observed, the sample is positive; if not, the sample is negative.		

Final result from combination of inhibition control and test reaction

IPC	GMO	Final result
Sample valid	Reaction positive	Positive
Sample valid	Reaction negative	Negative
Sample inhibited	Reaction positive	Positive
Sample inhibited	Reaction negative	Inhibited

7.2.1 Procedure

Evaluation of the IPC
Calculate the MEAN Ct (= Ct/Cp/Cq) value from NTC. Refer to data from IPC-detector. To calculate the Ct cut-off-1, subtract 3 Ct, to calculate the Cut-off-2 add 3 Ct.

Evaluation of 35S/NOS/FMV
Calculate the MEAN Ct (= Ct/Cp/Cq) value from positive control. Refer to data from 35S, FMV or nos detector, respectively. To calculate the Ct cut-off-1, subtract 10 Ct, to calculate the Cut-off-2, add 8 (for FMV: add 9) Ct. Calculate the MEAN Endpoint fluorescence value from positive control. Refer to data from 35S or nos detector, respectively. The Endpoint fluorescence cut-off is 20% of the MEAN Endpoint fluorescence.

Asymmetric target numbers for 35S/NOS/FMV have been validated. However, a competition of targets in a situation where highly different target numbers are present cannot be excluded.

Negative results in test reactions exhibiting

Ct / values **< Ct (C+) – 10 Ct**

in one or more PCR systems must be regarded as invalid, because they might have been caused by competition. The negative test results have to be confirmed with separate analyses.

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GMO*Screen* RT
35S/NOS/FMV IPC (LR) or (NR)

Test kit for real-time PCR detection of
CaMV 35S and FMV promoter and nos terminator
Cat. Nos: 5421220301 and 5421220302

7.2.2 Ambiguous results

If independently extracted DNAs show deviations in the results for “A” and “B”, this may be due to sample material non-homogeneity or to non-uniformities in the DNA-extraction efficiency. Repeat DNA extraction and homogenize sample more thoroughly. If the ambiguous result persists, it is most likely due to a GMO concentration close to the LOD.

8 PRODUCT WARRANTIES AND SATISFACTION GUARANTEE

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9 PRODUCT USE LIMITATIONS

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