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SpeciesScreen RT Plant IPC

Test kit for qualitative real-time PCR detection of plant DNA with internal positive control (IPC)

Cat. No. 5421229401

Includes reagents and control DNA for 96 real-time PCR reactions

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

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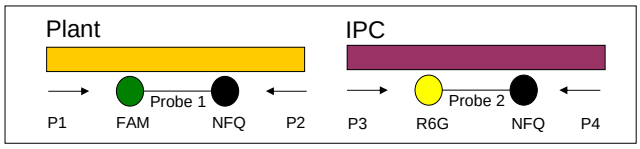
1 Introduction

The Species*Screen* Plant kit is a real-time PCR kit for detecting DNA from plants and can be used i.e. for detecting plant DNA extracted from food and feed.

The Species*Screen* RT Plant IPC kit is part of Eurofins GeneScan's comprehensive product portfolio for screening, identification and quantification of genetically modified organisms with the product lines Species*Screen,* Species*Ident,* GMO*Screen*, GMO*Ident,* and GMO*Quant*.

The first step in the detection and identification of plant DNA is the isolation of DNA from samples. The choice of the appropriate DNA isolation procedure is crucial and depends on the sample type.
We recommend the use of either our DNA*Extractor* kits (cat. no. 5224700610, -710) or our GENE*Spin* kit (cat. no. 5224400605).

The second step involves the detection of a specific DNA sequence by amplification with the real-time Polymerase Chain Reaction (RT-PCR).
The plant specific reaction amplifies a DNA sequence from the actin gene, which is specific for plant DNA in general.
A signal in the RT-PCR indicates that plant DNA is present in the sample.
An internal positive control (IPC) contained in the MasterMix is amplified in parallel and indicates inhibition for every sample, if present.



FAM™ and R6G are the fluorescent reporter dyes attached to the 5' ends of the probes for plant and the. Non-fluorescent quenchers are used for quenching.

2 Components of the Kit

The Species*Screen* Plant RT IPC kit contains reagents for a total of 96 PCR reactions including controls and specific reactions.
Store all reagents light protected at –20°C.

2x **Qualitative real-time MasterMix** Plant,

- 650 µL BasicMix QL RT (NR) GS-P-26.001
- 390 µL OligoMix QL RT IPC (LR/HR+)
  GS-P-09.103 • Plant actin

Mix prior to use in empty vial (labels included)!

2x **Genomic DNA Corn (non GM)** as positive control DNA (50 µL, 10 copies/µL)

1x **DNA stabilisation buffer**, 150 µL (for NTCs)

2x **ROX™ dye,** 23.4 µL (to be used only for High Rox (HR) cyclers, see chapter 6.3.1)

Important Note: Never store components of the kit together with samples or amplicons.
Never store or use them in areas where gel electro-phoresis or photo documentation is performed.

3 Preparation of the Kit's Reagents

Store the kit at –20°C until opened for the first time.
Thaw reagents just before use, mix them by vortexing and centrifuge briefly before use.
Just thaw as many BasicMix and OligoMix vials as necessary. Frequent freezing and thawing might cause inactivation of the reagents. If smaller volumes are needed, aliquot reagents before first use.
Refer to the reagent label for specific instructions regarding the correct storage.

4 Material and Equipment not included in the Kit

– Water, DNase-free
– Vortex
– Micropipettes (variable 1-1000 µL) and filter tips
– PCR optical tubes or plates 0.2 ml and optical caps or seals
– RT-PCR Thermocycler

5 Sample Preparation

5.1 DNA extraction

The extraction of DNA from the sample is a crucial step, and in order to guarantee optimal quantity and purity, an appropriate method has to be chosen for each sample matrix. For most products, the DNA can be extracted with a kit from our DNA*Extractor* kit line or with our GENE*Spin* kit.
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. 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.

Each sample should be treated in duplicate.

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 PCR

6.1 Special precautions during PCR analysis

PCR is an exponential reaction. In theory the detection of a single DNA target should be possible. The extreme sensitivity requires special precautions for handling and equipment. After a successful amplification several billion amplicons are present in the reaction tube. Each of them might lead to a false positive result when contaminating sample material, e.g. by spreading in aerosols.

The most important rules to avoid false-positive results are:

a) Separate the different procedures spatially. Use separate rooms for sample preparation, amplification and analysis of amplicons or at least dedicate

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different equipment and materials for each procedure. Mixing of PCR components should be done in a separate room, best on a clean bench. Keep all working steps separate from rooms where gel electrophoresis of amplicons is performed.

b) Use filter tips for micropipettes.

c) Wear disposable powder-free gloves.

d) Never store Species*Screen* Kits and materials for DNA extraction together with samples or amplicons.

Always perform extraction controls and PCR controls (NTCs).

6.2 General Information

PCR is performed in a volume of 25 µL in 0.2 mL reaction tubes/plates according to the RT-PCR cycler instructions.

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.

6.3 PCR Setup

6.3.1 Preparation of MasterMix

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.

Please be aware that the NTCs and positive controls (C+) are indispensable for the evaluation of the test and must be included in every run.

20 µL MasterMix for low ROX (LR) cyclers consist of 12.5 µL BasicMix + 7.5 µL OligoMix per reaction.

In order to provide MasterMix with high ROX (HR) level appropriate for laser real-time PCR systems (e.g. ABI 7900HT) ROX is added increasing the volume of the MasterMix to 20.45 µL.

20.45 µL HR MasterMix consist of 12.5 µL BasicMix + 0.45 µL ROX (50x) + 7.5 µL OligoMix per reaction.

The following reactions are required for a RT PCR run:

Samples and extraction controls	1	8	22	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
ROX (only for HR!)	*2.7 µL*	*9 µL*	*22.5 µL*	*43.2 µL*

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

6.3.2 Plate Setup

The following plate document 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 plus extraction control (EC); NTC = no template control; C+ = positive control; a and b = DNA extract duplicates

Every run requires 2 no template controls (NTC) and 2 positive controls (C+). It is highly recommended to test the samples in duplicate.

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6.3.3 Programming of the cycler

Before starting the practical work, program the plate document und the cycling conditions.
For description of the instrument programming please refer to the user manual of the respective instrument and software version.
For the plate template, you may use our templates for ABI 7500 and MX3000P/Mx3005P™ (send an e-mail mentioning your cycler model to kits@eurofins.com to receive a copy) or program your own template with the following settings:

ABI 7500 (7500 Fast), ABI 7900HT

System	Detector	Reporter	Quencher
plant	9103a	FAM™	NONE
IPC	9103i	VIC™	NONE
Passive Reference:		ROX™	

Agilent Mx3005P™ (Mx3000P™)

System	Assay	Filter
plant	9103a	FAM™/SYBR® Green I
IPC	9103i	VIC®/HEX™
ROX	ROX™	Texas Red®/ROX™

The fluorescence signals are scanned in the following order: (1) ROX™, (2) FAM™, (3) HEX™.

Roche LightCycler®480 Instrument I

Detection Format: Multi Color Hydrolyses Probe

System	Filter
plant	FAM (483-533)
IPC	VIC/HEX/Yellow555 (523-568)

On LC480, Color Compensation must be performed before the first test is run. Please use a Color Compensation kit (cat. # 5427200201)

6.3.4 Cycling Conditions

The run parameters are to be programmed as follows:

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

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

ABI 7900HT
Activate 9600 emulation mode.

6.4 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 Oligo Mix as explained 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, stabilisation 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, close lid 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: 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 RT-cyclers:** Set manually, 3-15
- **Agilent Mx3000P/Mx3005P™:** Adaptive

Roche LightCycler®480:
Choose “Abs Quant/2nd Derivative Max” and High Confidence settings to obtain Cp values.
Choose “Endpoint Genotyping” to obtain Endpoint Fluorescence.

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

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

CT Cut-offs	PLANT: Mean CT (C+) +6 IPC: Mean CT (NTC) +4
dR(n) Limits (EF for LC)	PLANT: Mean dR(n) (C+) x 0.2 IPC: Mean dR(n) (NTC) x 0.33
Outliers	Maximum acceptable outliers (C+): 0 Maximum acceptable outliers (NTC): 0

Inhibition control, scoring of IPC

CT and dR(n)	Results
CT_{IPC} sample ≤ CT_{IPC} Cut-off **and** $dR(n)_{IPC}$ sample ≥ $dR(n)_{IPC}$ Limit	valid
CT_{IPC} sample > CT_{IPC} Cut-off **or** $dR(n)_{IPC}$ sample < $dR(n)_{IPC}$ Limit	inhibited/invalid
No CT_{IPC}	inhibited/invalid
Only IPC	
Ct_{IPC} sample < Mean Ct_{IPC} NTC – 3	inhibited/invalid

Test reaction plant

CT	dR(n) (for LC:EF)	Results
CT_{PLANT} sample ≤ CT_{PLANT} Cut-off	$dR(n)_{PLANT}$ sample ≥ $dR(n)_{PLANT}$ Limit	Reaction positive
CT_{PLANT} sample ≤ CT_{PLANT} Cut-off	$dR(n)_{PLANT}$ sample < $dR(n)_{PLANT}$ Limit	*Check amplification!*
CT_{PLANT} sample > CT_{PLANT} Cut-off	$dR(n)_{PLANT}$ sample ≥ $dR(n)_{PLANT}$ Limit	Reaction positive
CT_{PLANT} sample > CT_{PLANT} Cut-off	$dR(n)_{PLANT}$ sample < $dR(n)_{PLANT}$ Limit	Reaction negative
No CT_{PLANT}	-	Reaction negative
***Note*:** In case of "Check amplification!" the amplification plots must be checked visually for presence of a sigmoid PCR amplification signal.		

Final result from combination of inhibition control and test reaction

IPC	PLANT	Final result
valid	Reaction positive	Positive
valid	Reaction negative	Negative
inhibited/invalid	Reaction positive	Positive
inhibited/ invalid	Reaction negative	Inhibited

Evaluation of the IPC
Calculate the MEAN Ct Value from NTC. Refer to data from IPC-detector. To calculate the Ct cut-off, add 4 Ct, to calculate the 2nd Ct cut-off, subtract 3 Ct.
Calculate the MEAN dR(n) Value from NTC. Refer to data from IPC-detector. The dR(n) cut-off is 33% of the MEAN dR(n).

Evaluation of PLANT
Calculate the MEAN Ct Value from positive Control. To calculate the Ct cut-off, add 6 Ct. Calculate the MEAN dR(n) value from positive control. The dR(n) cut-off is 20% of the MEAN dR(n).

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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 PLANT concentration close to the LOD.

8 Limit of detection, quantification

The absolute limit of detection (LOD_{abs}) for the method has been validated as ≤ 10 copies of plant target per reaction.
The limit of detection for the analysis of individual samples depends on several factors: the presence of PCR-inhibiting substances, the capability of the DNA extraction method to remove these inhibitors and the degree of DNA damage, and thus strongly on the type of sample and the DNA extraction procedure.

Quantification is not possible with this kit.

9 PRODUCT USE LIMITATIONS

This Eurofins GeneScan Technologies 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 the materials described in this text.

10 Important Notes

- The PCR and TaqMan processes are covered by patents issued to Hoffmann-La Roche AG and Applied Biosystems Inc., which are applicable in certain countries.
- GeneScan Technologies does not encourage or support the unauthorized or unlicensed use of this process. Use of these products is recommended for persons that either have a license to perform PCR or are not required to obtain a license.
- Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

11 Troubleshooting

Result	Possible mistakes/reasons	Possible verification and measures
No PCR signals from samples	Inhibition of PCR by inhibitory substances. Inhibition by too much DNA.	Clean DNA further* or dilute DNA solution. Too much DNA inhibits the PCR. Check dilution.
No PCR signals from positive controls	Wrong PCR program. MasterMix not prepared correctly.	Check and correct PCR program. Check and correct preparation of MasterMix.

* Repeat extraction of DNA from the sample. Repeat washing with 75 % ethanol three times. If necessary, clean DNA further e.g. with our DNA cleaning columns (cat. no. 5224700310).

12 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 of 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.

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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.

13 TECHNICAL SERVICE

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. GeneScan Technologies customers are 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 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 the GeneScan Technologies Technical Service Department or local distributors.