

CONGEN

SureFood® GMO SCREEN 4plex 35S/NOS/FMV+IAC

Art. No. S2126
100 rxn

User Manual



March 2023

 Inhalt

1	Allgemeines	3
1.1	Beschreibung	3
1.2	Nachweisgrenze	3
1.3	DNA-Präparation	4
1.4	Kit-Inhalt und Lagerung	4
1.5	Zusätzliche benötigte Geräte und Materialien	4
1.6	Geräteeinstellungen	5
1.7	Detektionskanaleinstellungen	5
2	Qualitative Analyse	6
2.1	Protokoll	6
2.1.1	Herstellen des Master-Mix	6
2.1.2	Herstellen des real-time PCR-Mix	6
2.2	Interpretation der Ergebnisse	7
3	Weitere Informationen	8
3.1	Weitere Dokumente und Hilfsmittel	8
3.2	Technischer Support	8
3.3	Vertrieb und Bestellung	8



Content

1	General Information	9
1.1	Description	9
1.2	Limit of Detection	9
1.3	DNA-preparation	10
1.4	Kit components and storage	10
1.5	Additionally required equipment and materials	10
1.6	Setup.....	11
1.7	Detection channel Set-up	11
2	Qualitative Analysis	12
2.1	Protocol	12
2.1.1	Preparation of the master-mix	12
2.1.2	Preparation of the real-time PCR-mix	12
2.2	Interpretation of results	13
3	Further Information	14
3.1	Product Information.....	14
3.2	Technical Support	14
3.3	Distribution and Ordering	14

1 General Information

1.1 Description

The SureFood® GMO SCREEN 4plex 35S/NOS/FMV+IAC is a real-time PCR for the direct, qualitative detection and differentiation of following specific DNA sequences.

- 35S promoter of Cauliflower Mosaic Virus (CaMV),
- NOS terminator of the soil bacterium *Agrobacterium tumefaciens*
- 34S FMV promoter from figwort mosaic virus

This kit can be used for screening of genetically modified organisms (GMOs) in food, feed and seeds.

The detections are according to the official collection of detection methods of §64 German food law.

Each reaction contains an internal amplification control (IAC). If the DNA contains PCR inhibiting substances, the signal of the amplification control will be affected or the amplification will be suppressed. Examples for PCR inhibiting substances are alcohols (e.g. ethanol, isopropanol), surfactants (e.g. CTAB, SDS, Triton X100) and salts (e.g. sodium chloride). In addition spices, herbs, algae, cocoa and further sample matrices might have PCR inhibiting effects.

The real-time PCR assay can be performed with commonly used real-time PCR instruments, equipped for detection of four fluorescence emissions at the channels FAM, VIC/HEX, ROX and Cy5 at the same time. The technical verification of instruments was performed on Roche LightCycler® 480 II, Qiagen Rotor-Gene Q, R-Biopharm RIDA®CYCLER, Bio-Rad CFX96, Bio-Rad CFX96DX, Bio-Rad CFX Opus 96 and Agilent AriaDx.

1.2 Limit of Detection

The SureFood® GMO SCREEN 4plex 35S/NOS/FMV+IAC real-time PCR has a limit of detection of ≤ 5 DNA copies.

The assay limit of detection depends on sample matrix, processing grade, DNA preparation and DNA content.

The SureFood® PCR systems are very sensitive and therefore even a small amount of target DNA is sufficient for a successful analysis. The concentration of total DNA in the sample does not allow a conclusion on the quantity and quality of the target DNA.

Note: Inconsistent mixing ratios* may cause a loss of sensitivity in the low concentration channel in mixed samples especially with high amplicon concentrations (Cp value < 20).

* e.g. 99.9 Roundup Ready Soya (35S, NOS) and 0.1 % Roundup Ready 2 Yield Soya (FMV)

SureFood® GMO SCREEN 4plex 35S/NOS/FMV+IAC (100 rxn)

Art. No. S2126

March 2023

1.3 DNA-preparation

For DNA-preparation of raw material the use of SureFood® PREP Basic (Art. No. S1052), SureFast® Mag PREP Food (Art. No. F1060) and for highly processed food and feed the use of SureFood® PREP Advanced (Art. No. S1053) is recommended. SureFood® PREP Add On (Art. No. S1055) is intended to be used for the extraction of DNA from raw materials as well as processed food and feed with sample weight of 2 g. It is used in conjunction with the SureFood® PREP Basic.

1.4 Kit components and storage

Kit Code	Reagent	Amount	Lid Color
1	Reaction Mix	2 x 1050 µl	Yellow
2	Taq Polymerase	1 x 80 µl	Dark Red
3	Positive Control	1 x 190 µl	Light Blue

Store all reagents at –20°C and protected from light. The Taq Polymerase can be stored at +2 to +8°C for multiple uses on the same day.

Note: The Taq Polymerase may be in a frozen or unfrozen state. This does not affect the quality of the Taq Polymerase or the performance of the real-time PCR.

1.5 Additionally required equipment and materials

- DNA-Extraction kit
(e.g. SureFood® PREP Basic Art. No. S1052 / SureFood® PREP Advanced Art. No. S1053 / SureFood® PREP Add On Art. No. S1055 / SureFast® Mag PREP Food Art. No. F1060))
- real- time PCR instrument with four detection channels (510 nm, 580 nm, 610 nm and 660 nm)
- real-time PCR consumable (plates, tubes, foils, caps)
- pipettes with filter tips
- powder-free disposable gloves
- Vortex mixer
- micro centrifuge with a rotor for the reaction tubes

SureFood® GMO SCREEN 4plex 35S/NOS/FMV+IAC (100 rxn)

Art. No. S2126

March 2023

1.6 Setup

	Blockcycler & R-Biopharm RIDA®CYCLER	Rotorcycler
Initial Denaturation (HOLD)	5 min, 95°C	1 min, 95°C
Cycles	45	45
Denaturation	15 sec, 95°C	10 sec, 95°C
Annealing/Extension (CYCLE)	30 sec, 60°C	15 sec, 60°C
Temperature Transition Rate/ Ramp Rate	Maximum	Maximum

1.7 Detection channel Set-up

Real-time PCR device	Detection	Detection channel	Quencher	Note
Agilent AriaMx / Dx	35S	FAM	+	
	IAC	HEX	+	
	FMV	ROX	+	
	NOS	Cy5	+	
Qiagen Rotor-Gene Q	35S	green	+	Note: Please use only 0.1 ml reaction tube. The gain settings must be set to 5 (factory default) for all channels.
	IAC	yellow	+	
	FMV	orange	+	
	NOS	red	+	
Bio-Rad CFX96 / Dx / CFX Opus 96	35S	FAM	+	
	IAC	VIC/HEX	+	
	FMV	ROX	+	
	NOS	Cy5	+	
R-Biopharm RIDA®CYCLER	35S	green	+	
	IAC	yellow	+	
	FMV	orange	+	
	NOS	red	+	
Roche LightCycler® 480 II	35S	465-510	+	The SureCC Color Compensation Kit I (Art. No. F4009) is required.
	IAC	533-580	+	
	FMV	533-610	+	
	NOS	618-660	+	

2 Qualitative Analysis

2.1 Protocol

2.1.1 Preparation of the master-mix

Calculate the total number of reactions needed (samples and control reactions) for the specific PCR assay as well as for the inhibition control.

Recommended control reactions for the specific PCR assay: negative control, extraction control, positive control. The reaction mix contains an internal amplification control (IAC) per reaction.

Reactions needed for the qualitative 35S, NOS and FMV detection:

3 reactions for controls (1x no-template control, 1x extraction control, 1x positive control)

For each sample: at least 1 reaction for each sample DNA

It is also recommended to prepare the master-mix with 10 % additional volume in order to compensate reagent loss. Allow the reagents to thaw, mix and centrifuge before opening and use.

Example for the calculation and preparation of 10 reactions:

Components of the master-mix	Amount per reaction	10 reactions (with 10% excess)
Reaction Mix	19.3 µl	212.3 µl
Taq Polymerase	0.7 µl	7.7 µl
Total volume	20 µl	220 µl

Mix each master-mix well and centrifuge shortly before use.

2.1.2 Preparation of the real-time PCR-mix

- Pipette 20 µl of the master-mix into appropriate tubes/wells.
- Close the negative control (the negative control is ready for PCR without any addition).
- Pipette 5 µl of sample DNA into the designated tubes/wells and close them.
- Pipette 5 µl of Positive Control into the designated tubes/wells and close them.
- Centrifuge all tubes/plates shortly at low speed.
- Place tubes/plates into the real-time PCR instrument and start the run according to the setup.

2.2 Interpretation of results

The evaluation has to be made according to the usual analysis program recommended by the real-time PCR instrument manufacturer.

The control reactions have to show the correct results.

35S DNA is detected in the FAM-channel, FMV DNA is detected in the ROX-channel and NOS DNA is detected in the Cy5-channel (see table). In the VIC/HEX-channel the amplification control is detected.

A sample is stated **positive** for the respective parameter, if the sample DNA shows amplification in the respective channel. High amplicon concentrations can result in a weak or absent signal of the internal amplification control (IAC).

A Cp value for the internal amplification control (IAC) is not needed to obtain a positive result of the positive control.

A sample is stated **negative** for the respective parameter, if the sample DNA shows no amplification in the respective channel and if the internal control (VIC/HEX-channel) of the sample is **positive** with a shift in Cp-value ≤ 2 compared to the negative control. If the sample DNA in the VIC/HEX-Channel shows **no amplification** or a shift in Cp-value > 2 compared to the negative control, it contains PCR inhibiting substances. A significant decrease in the fluorescence signal can also show the presence of PCR inhibiting substances. Under these circumstances' DNA isolation and purification of the sample need to be improved. Alternatively, the DNA can be diluted (recommendation 1:2 in PCR-water) and analysed again for inhibition. Please note that the dilution factor also affects the detection limit of the specific 35S, NOS or FMV PCR assay.

It may appear in some cases that only one of the two DNA duplicates prepared from the test sample is 35S and/or NOS and/or FMV **positive**. This indicates that the amount of genetically modified DNA is very low and at the limit of detection. If such results are obtained in the analysis (see DIN EN ISO 24276:2013-10), the sample is stated negative.

result in the respective channel				Interpretation
FAM channel 35S	ROX channel FMV	Cy5 channel NOS	VIC/HEX channel IAC	
positive	negative	negative	positive/negative	35S DNA detected
negative	positive	negative	positive/negative	FMV DNA detected
negative	negative	positive	positive/negative	NOS DNA detected
positive	negative	positive	positive/negative	35S/NOS DNA detected
positive	positive	positive	positive/negative	35S/NOS/FMV DNA detected
negative	negative	negative	positive	Negative, target DNA is not detected
negative	negative	negative	negative	invalid

SureFood® GMO SCREEN 4plex 35S/NOS/FMV+IAC (100 rxn)

Art. No. S2126

March 2023

Note: The 35S promoter is originally derived from the Cauliflower Mosaic Virus. *Brassicaceae* species are the host of the CaMV. Therefore, the analysis for the 35S promoter also detects the naturally occurring virus. It is possible that a sample containing the virus yields a positive result, although no GMO is present. As a supplementary kit, please use the SureFood® GMO SCREEN CaMV kit (Art. No. S2027), which helps to assure the absence of the natural Cauliflower Mosaic Virus in the sample in case of a single positive 35S result.

3 Further Information

3.1 Product Information

- Detailed information about setup of several real-time PCR devices
(Download: www.congen.de/en/company/downloads)
- Validation Report upon request

3.2 Technical Support

For further questions please contact your distributor or send an e-mail to sales@r-biopharm.de.

3.3 Distribution and Ordering

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