CONGEN

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

Art. No. S2126
100 rxn

User Manual

August 2025

Content

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 internal technical verification of instruments was performed on Roche LightCycler® 480 II, Agilent AriaDx, Qiagen Rotor-Gene Q, Bio-Rad CFX96 Dx, Bio-Rad CFX96 Opus and R-Biopharm RIDA®CYCLER.

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 < 27).

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

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 samples 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 -28 to -16°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

1.6 Precautions for users

- Extraction, PCR preparation and the PCR run should be separated in different rooms to avoid cross-contaminations.
- This test must only be performed by laboratory personnel trained in molecular biology methods.
- Strictly follow the working instructions.
- When handling samples, wear disposable gloves. After finishing the test, wash your hands.
- Do not smoke, eat or drink in areas where samples or test reagents are being used.
- Do not use the kit after the expiration date.
- All reagents and materials used have to be disposed properly after use. Please refer to the relevant national regulation for disposal.

1.7 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

1.8 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	None	**Note:** Please use only 0.1 ml reaction tube. The gain settings must be set to 5 (factory default) for all channels.
	IAC	yellow	None	
	FMV	orange	None	
	NOS	red	None	
Bio-Rad CFX96/Dx/Opus	35S	FAM	+	Baseline Settings: • Baseline substracted curve fit • Apply fluoresnce drift correction
	IAC	VIC/HEX	+	
	FMV	ROX	+	
	NOS	Cy5	+	
R-Biopharm RIDA®CYCLER	35S	green	+	**Ignore cycles before**, if there is a significant deviation in the baseline at the start of the run. Please see page 45 of the cycler operating instructions, section 12.1.2 Cycling analysis parameter.
	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.

The following control reactions are needed for the specific PCR assay: negative control, extraction control, Positive Control.

The reaction mix contains an internal amplification control (inhibition control) per reaction.

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

3 reactions for controls***** (1x negative 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.
- 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.

*** Description of the controls**

- Negative control: only master-mix
- Extraction control: the extraction is performed without the sample – components from used Prep Kit
- Positive Control: master-mix and within the kit's provided Positive Control

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 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 PCR assay.

It may appear in some cases that only one of the two DNA duplicates prepared from the test sample is **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 at least two repetitions of the analysis, 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

Note: The results displayed in the table above represent merely an example. Additional combinations are also possible.

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.

The following table shows the specification ranges of the kit controls

	Specification range	Internal amplification control (IAC)
negative control	negative	25 ≤ Cp ≤34
Positive Control (FAM –35S)	25 ≤ Cp ≤33	not relevant
Positive Control (ROX –FMV)	25 ≤ Cp ≤33	not relevant
Positive Control (CY5 –NOS)	25 ≤ Cp ≤33	not relevant

3 Limitations of the method

- The presence of PCR inhibitors may cause invalid results.
- Extremly low levels of target below the limit of detection (LoD) may be detected, but results may not reproducible.
- In highly processed samples, the detection limit may be shifted. Factors such as high pressures, mechanical stresses, chemical treatment, extreme temperatures and/or extreme pH values during manufacturing process – such as in canning production – can damage or degrade nucleic acids. This means that the sensitivity of the test kit may be reduced and not all original components may be detected.

4 Further Information

4.1 Product Information

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

4.2 Technical Support

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

4.3 Distribution and Ordering

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