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SureFood® GMO SCREEN 4plex BAR/NPTII/PAT/ CTP2:CP4 EPSPS

Art. No. S2127
100 rxn

User Manual

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Content

1 General Information

1.1 Description

The SureFood® GMO SCREEN 4plex BAR/NPTII/PAT/CTP2:CP4 EPSPS is a real-time PCR for the direct, qualitative detection and differentiation of following specific DNA sequences.

- Phosphinothricin-Acetyltransferase gene (BAR) from *Streptomyces hygroscopius*
- Antibiotics-resistance gene Neomycin-Phosphotransferase (nptII)
- Phosphinothricin-Acetyltransferase gene (PAT) from *Streptomyces viridiochromogenes*
- the transition from CTP2 (Chloroplast-Transpeptide-signal sequence from *Arabidopsis thaliana*) to herbicide tolerance-gene CP4 EPSPS (5-Enolpyruvylshikimat-3-Phosphat Syntheses gene from *Agrobacterium tumefaciens* strain CP4)

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 and Feed Code (LFGB), especially according to technical specification BVL L-00.00-154.

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 Agilent AriaDx, Bio-Rad CFX96 Dx, Bio-Rad CFX Opus 96, Qiagen Rotor-Gene Q, R-Biopharm RIDA®CYCLER, and Roche LightCycler® 480 II.

1.2 Limit of Detection

The SureFood® GMO SCREEN 4plex BAR/NPTII/PAT/CTP2:CP4 EPSPS 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% MON88017 Corn and 0.1 % Bt176 Corn

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 AriaDx /Mx	nptII	FAM	+	
	PAT, moPAT	HEX	+	
	CTP2:CP4 EPSPS	ROX	+	
	BAR	Cy5	+	
Bio-Rad CFX96/Dx/Opus	nptII	FAM	+	Baseline Settings: • Baseline substracted curve fit • Apply fluoresnce drift correction
	PAT, moPAT	VIC/HEX	+	
	CTP2:CP4 EPSPS	ROX	+	
	BAR	Cy5	+	
Qiagen Rotor-Gene Q	nptII	green	+	**Note:** Please use only 0.1 ml reaction tube. The gain settings must be set to 5 (factory default) for all channels.
	PAT, moPAT	yellow	+	
	CTP2:CP4 EPSPS	orange	+	
	BAR	red	+	
Roche LightCycler® 480 II	nptII	465-510	+	The SureCC Color Compensation Kit I (Art. No. F4009) is required.
	PAT, moPAT	533-580	+	
	CTP2:CP4 EPSPS	533-610	+	
	BAR	618-660	+	
R-Biopharm RIDA®CYCLER	nptII	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.
	PAT, moPAT	yellow	+	
	CTP2:CP4 EPSPS	orange	+	
	BAR	red	+	

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 and an external inhibition control per sample.

For the preparation of the inhibition control the use of the SureFood® GMO Plant PLUS (Art. No. S2049) is recommended.

Reactions needed for the qualitative BAR, nptII, PAT/moPAT and CTP2:CP4 EPSPS 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.

NptII DNA is detected in the FAM-channel, PAT/moPAT DNA is detected in the VIC/HEX-channel, CTP2:CP4 EPSPS DNA is detected in the ROX-channel and BAR DNA is detected in the Cy5-channel (see table).

A sample is stated **positive** for the respective parameter, if the sample DNA shows amplification in the respective channel.

A sample is stated **negative** for the respective parameter, if the sample DNA shows no amplification in the respective channel and if the external inhibition control of the sample is **positive** with a shift in Cp-value ≤ 2 compared to the negative control.

If the sample DNA in the external inhibition control 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				Result	Interpretation
FAM channel nptII	VIC/HEX channel PAT/moPAT	ROX channel CTP2:CP4 EPSPS	Cy5 channel BAR	external inhibition-control	
positive	negative	negative	negative	**positive**	nptII DNA detected
negativ	**positive**	negative	negative	**positive**	PAT/moPAT DNA detected
negativ	negative	**positive**	negative	**positive**	CTP2:CP4 EPSPS DNA detected
negativ	negative	negative	**positive**	**positive**	BAR DNA detected
negativ	negative	negative	negative	**positive**	Negative, target DNA is not detected
negativ	negative	negative	negative	negative	invalid

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

The following table shows the specification ranges of the kit controls

	Specification range
Positive Control (FAM – nptII)	25 ≤ Cp ≤33
Positive Control (VIC – PAT/moPAT)	25 ≤ Cp ≤33
Positive Control (ROX – CTP2:CP4 EPSPS)	25 ≤ Cp ≤33
Positive Control (CY5 – BAR)	25 ≤ Cp ≤33

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 limit of detection 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

R-Biopharm AG
An der neuen Bergstrasse 17,
64297 Darmstadt, Germany
Phone: +49 (0) 61 51 - 81 02-0
Fax: +49 (0) 61 51 - 81 02-20
E-Mail: orders@r-biopharm.de
www.r-biopharm.com