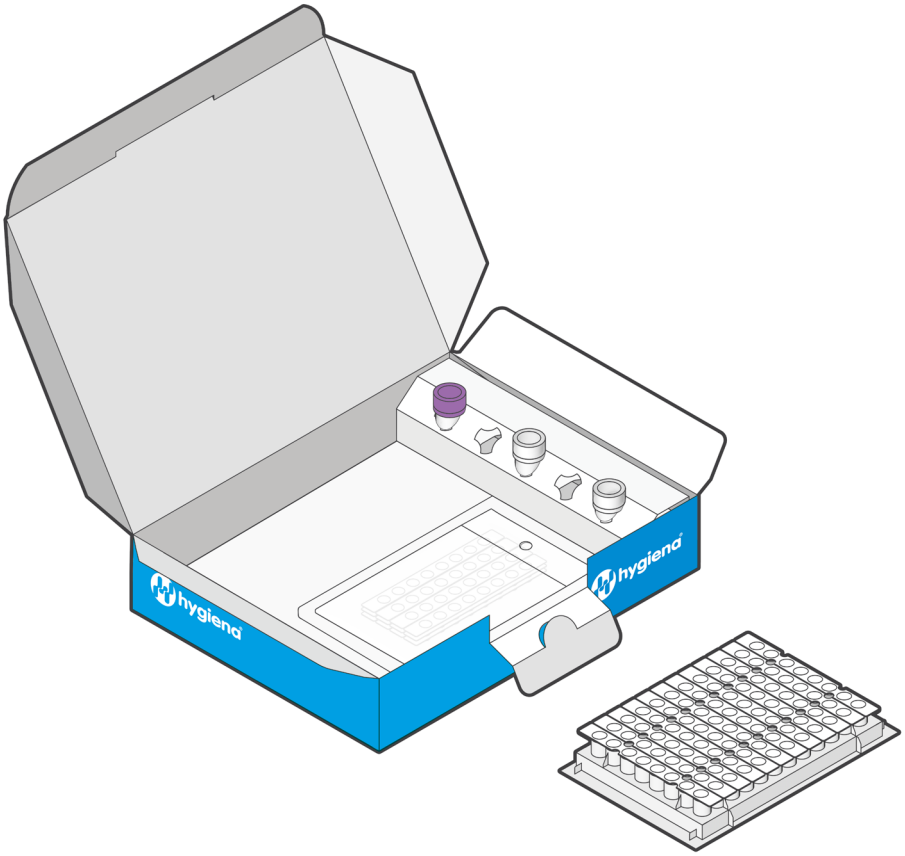


foodproof®

Aspergillus Detection LyoKit

PRODUCT INSTRUCTIONS

Documentation for the qualitative detection of
Aspergillus flavus, *Aspergillus terreus*,
Aspergillus niger and *Aspergillus fumigatus*

Product Nos. KIT230145 / KIT230146

foodproof®

Aspergillus Detection LyoKit

Product No.

LP: KIT230145

RP: KIT230146

Kit for 96 reactions (lyophilized) for a maximum of 94 samples

Store kit at 2 °C to 8 °C

For testing of food, hemp and cannabis samples

PRODUCT INSTRUCTIONS

Revision A, February 2025

TABLE OF CONTENTS

1. OVERVIEW

1.1 General Information

Number of Reactions

The kit is designed for 96 reactions. Up to 94 samples plus positive and negative control can be analyzed per run.

Storage and Stability

Store all components at 2 to 8 °C. They are guaranteed to be stable through the expiration date printed on the label. Opening of the kit does not shorten the expiration date. The PCR strips must be stored in the provided aluminum bag. Protect from light and moisture.

LyoKit Tube Profiles

The LyoKit is available in two different tube profiles: white low profile tubes (LP) and clear regular profile tubes (RP).

The majority of real-time PCR cyclers use low profile tubes (LP). For the BAX® Q7 System, please use LP tubes/kit only. For a detailed overview, please have refer to our compatibility chart on www.hygiena.com.

1.2 Applicability

The foodproof® *Aspergillus* Detection LyoKit is intended for the rapid detection of *Aspergillus flavus*, *A. terreus*, *A. niger* and *A. fumigatus* DNA, isolated from enrichment cultures prepared by valid methods with all relevant kinds of samples that are potentially contaminated with these microorganisms, e.g., food, feed or cannabis samples. *A. flavus* and *A. terreus* are detected in individual channels, while *A. niger* and *A. fumigatus* can be differentiated by melting curve analysis (optional).

The foodproof *Aspergillus* Detection LyoKit is designed for the food, feed, cannabis and hemp testing laboratories. The kit must not be used in diagnostic procedures.

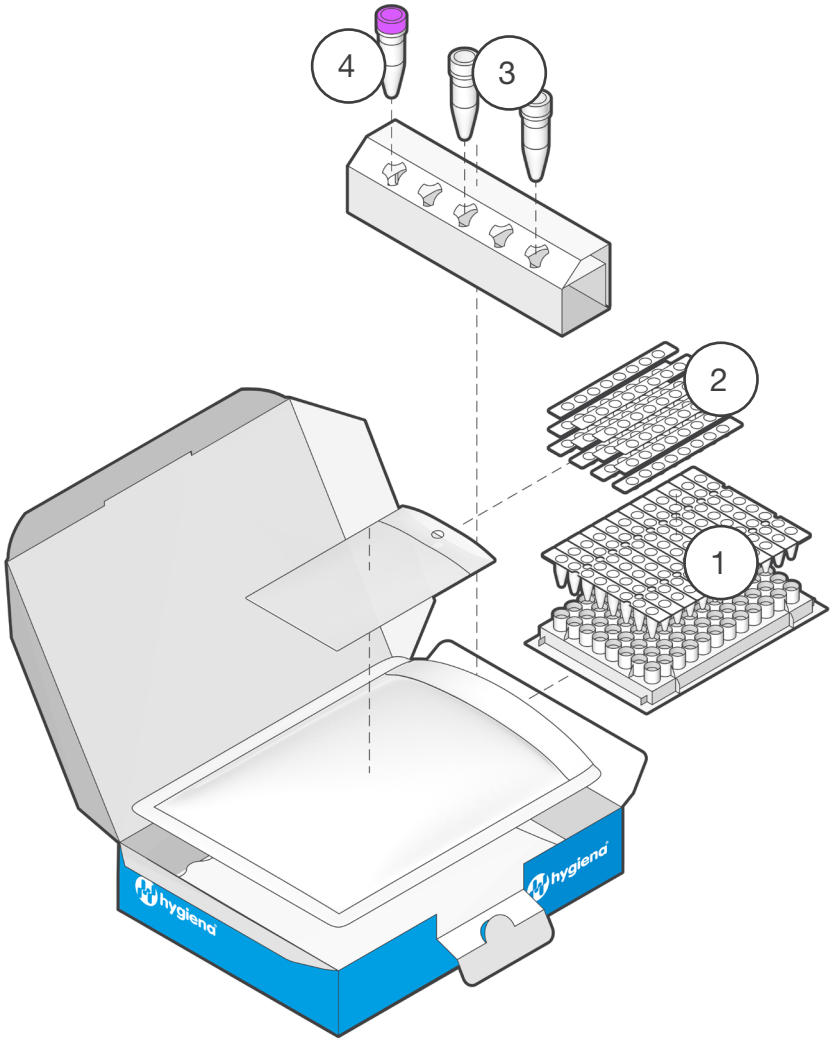
The kit described in this instruction manual has been developed for real-time PCR instruments with a FAM, a VIC/Yakima Yellow or HEX, a ROX or Texas Red and a Cy5 detection channel, and capable of performing a melting curve analysis (optional).

The performance of the kit was tested with the following real-time PCR instruments: LightCycler® 480, LightCycler® 96 (Roche Diagnostics), Mx3005P®, AriaMx (Agilent Technologies), Applied Biosystems™ 7500 Fast, QuantStudio™ 5 (Thermo Fisher Scientific), CFX96™ (Bio-Rad), BAX® Q7 System and Dualo 32® R2 (Hygiena).

1.3 Kit Contents

A schematic representation of the foodproof *Aspergillus* Detection LyoKit with all its components.

LP: KIT230145
RP: KIT230146



	Component	Details
1	Microplate	12 x 8-tube strips, prefilled with lyophilized ready-to-use PCR mix. Available are different tube profiles: white low profile tubes (LP) and clear regular profile tubes (RP).*
2	12 x 8-cap strips	For use in real-time PCR after addition of samples.
3	2 x H_2O PCR-grade (colorless cap)	1 mL nuclease-free, for use as a PCR run negative control.
4	Control Template (purple cap)	300 µL, contains a stabilized solution of DNA for use as a PCR run positive control.

* Tube profile and instrument compatibility chart is available online

2. INSTRUCTIONS

2.1 Required Material

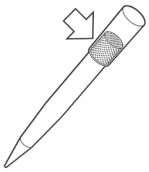
Most of the required equipment and reagents are available through Hygiena®.
Please contact us for further information.

Use a real-time PCR cycler suitable for detection of respective probes as well as for using low or regular profile strip tubes.
In case the strip tubes don't fit for the instrument, the samples should be transferred to appropriate PCR vessels after resuspension of the lyophilized PCR mix.

Important note: To use the foodproof Aspergillus Detection LyoKit with the BAX Q7 and the BAX®Prep Lysis methods, please use the separately available instructions for the foodproof Aspergillus with BAX®Prep Lysis Kit (KIT2046).

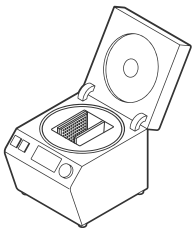
Material

☐ Nuclease-free, aerosol-resistant pipette **filter tips**.



☐ **PCR strip or plate centrifuges**

- *Without vortex: Mini microcentrifuge for 4 x 8-strips*
- *With vortex: Multispin MSC-6000 for 4 x 8-strips*
- *With vortex: CVP-2 for 12 x 8-strips and plates*



2.2 Precautions and Preparations

The kit provides all reagents required for the PCR. However, in order to achieve reliable results, the entire assay procedure must be performed under nuclease-free conditions. Follow the instructions below to avoid nucleases, carry-over or cross-contamination:

- ☐ Keep the kit components separate from other reagents in the laboratory.
- ☐ Use nuclease-free labware (e.g., pipettes, pipette tips, reaction vials).
- ☐ Wear gloves when performing the assay.
- ☐ To avoid cross-contamination of samples and reagents, use fresh aerosol barrier pipette tips.
- ☐ To avoid carry-over contamination, transfer the required solutions for one experiment into a fresh tube, rather than directly pipetting from stock solutions.
- ☐ Physically separate the workplaces for DNA preparation, PCR setup and PCR to minimize the risk of carry-over contamination. Use a PCR hood for all pipetting steps.
- ☐ **Sample Material**: Use any sample material suitable for PCR in terms of purity, concentration and absence of inhibitors.
- ☐ **DNA Extraction**: We provide sample preparation kits suitable for all kind of food and other samples.
- ☐ **Positive Control**: Always run a positive control with the samples. Use the provided control DNA (Control Template) or a positive sample preparation control.
- ☐ **Negative Control**: Always run a negative control with the samples. To prepare a negative control, replace the template DNA with PCR-grade water. Include a negative control during sample preparation to monitor reaction purity and cross-contamination. This extraction control can be used as an additional negative control reaction.
- ☐ **Confirmation**: If required, positive results may be confirmed by appropriate methods (e.g., reference method).
- ☐ **Waste Disposal:** All contaminated and potentially infectious material, like enrichment cultures or food samples, should be autoclaved before disposal and eliminated according to local rules and regulations. For more information, e.g., proper disposal of unused chemicals, please refer to the appropriate safety data sheet (SDS).

Keep the PCR mix away from light and moisture.

For more information, please refer to the appropriate safety data sheet (SDS). The SDS is available online at www.hygiena.com/sds.

2.3 Enrichment and DNA extraction

For Hemp flowers and cannabis flowers, combine 10 g test portion with 90 ml buffered peptone water (BPW) with 100 mg/L chloramphenicol and incubate at 37 °C for 44 hours.

For DNA Extraction, use the foodproof StarPrep Two *Aspergillus* Kit (KIT230177) and follow the instructions for DNA isolation using the StarPrep Two - *Aspergillus* Kit.

To use the foodproof *Aspergillus* Detection LyoKit with the BAX Q7 system and the BAX®Prep Lysis method, please refer to the instructions for the foodproof *Aspergillus* with BAX®Prep Lysis Kit (KIT2046).

Note: This kit was successfully tested with cannabis and hemp matrices like oils, chocolate, tea, coffee, creams, shampoo, flowers and seeds.

2.3.1 Methodology

When using the foodproof *Aspergillus* Detection LyoKit, DNA extraction/lysis was performed using either the BAX®Prep *Aspergillus* Lysis Kit or the foodproof StarPrep Two Kit.

Important note: To use the foodproof *Aspergillus* Detection LyoKit with the BAX Q7 and the BAX®Prep Lysis methods, please use the separately available instructions for the foodproof *Aspergillus* with BAX®Prep Lysis" Kit (KIT2046).

Detection Channels:

All *Aspergillus flavus* strains were detected in FAM channel, all *Aspergillus terreus* in HEX/VIC channel and all *Aspergillus niger* and *Aspergillus fumigatus* in ROX channel. None of the non-target strains were detected in any channel.

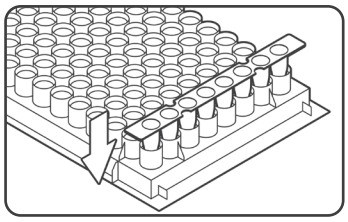
Sensitivity of Detection:

A relative detection limit of 1 to 10 cells per 25/100 g sample can be achieved with most kinds of foods. The foodproof *Aspergillus* Detection LyoKit detects down to 10^2 - 10^4 CFU/ml of *Aspergillus flavus*, *Aspergillus terreus*, *Aspergillus niger* or *Aspergillus fumigatus* enrichment culture (depending on the sample preparation kit used).

2.4 Procedure

This protocol describes how to perform the analysis of DNA extracted with foodproof StarPrep Two by real-time PCR. ***Important Note: For use with BAX Q7 and BAX®Prep Lysis methods, please use the separately available instructions for the foodproof Aspergillus with BAX®Prep Lysis Kit, KIT2046.***

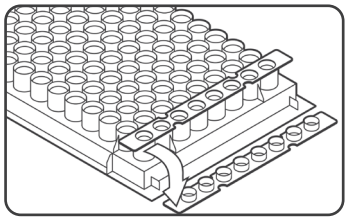
2.4.1 Workflow



1. PLACE STRIPS IN RACK

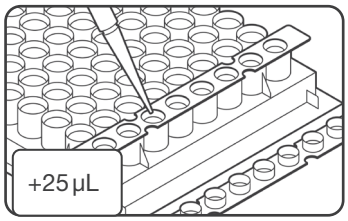
Take needed number of PCR tube strips out of aluminum bag.
Important: tightly seal bag afterward. Place strips in a suitable PCR tube rack.

If needed, gently tap the tubes to move the lyophilized pellets to the bottom of all tubes.



2. UNCAP

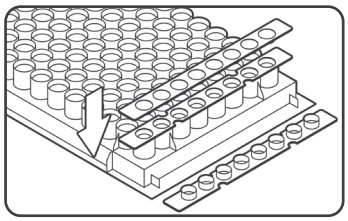
Open strips carefully direct before filling and discard caps.
Important: do not leave open longer than necessary.



3. ADD SAMPLES AND CONTROLS

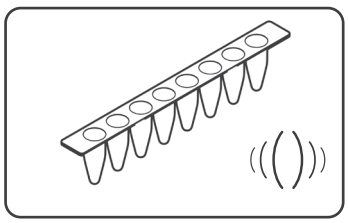
Pipette 25 µL of samples, negative control (colorless cap) or Control Template (purple cap) into respective wells.

If using less volume, add PCR-grade H_2O to reach 25 µL.
To reduce the risk of cross-contamination, prepare only one strip at a time.



4. SEAL

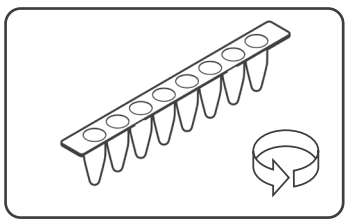
Seal the tubes with the provided 8-cap strips tightly.



5. MIX

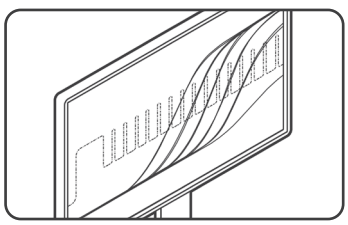
Resuspend pellet after sealing by mixing thoroughly.

Alternatively, resuspend pellet by pipetting up and down multiple times in step 3.



6. CENTRIFUGE

Briefly spin strips, e.g., 5 sec at 500 - 1,000 x g, in a suitable centrifuge.



7. START REAL-TIME PCR RUN

Cycle samples as described in the program setup (2.4.2). Place tubes in a vertical, balanced order into the cycler,
e.g., two strips can be placed in the first and last columns.

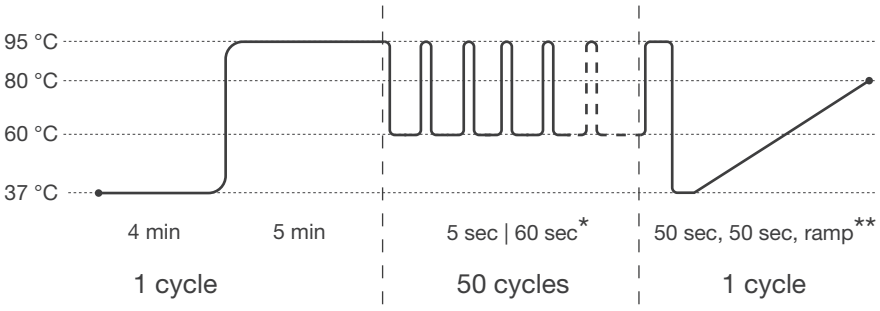
2.4.2 Program Setup

For PCR Detection on instruments other than the BAX System Q7.

Important note: To use the foodproof Aspergillus Detection LyoKit with the BAX Q7 and the BAX®Prep Lysis methods, please use the separately available instructions for the foodproof Aspergillus with BAX®Prep Lysis Kit, KIT2046.

Program your real-time PCR instrument before setting up the PCR reactions. Select the following channels:

▶ FAM (*A. flavus*), HEX (*A. terreus*), ROX (*A. niger* and *A. fumigatus*), and Cy5 (Internal Control).



Pre-incubation: 1 cycle
Step 1: 37 °C for 4 min
Step 2: 95 °C for 5 min

Amplification: 50 cycles
Step 1 : 95 °C for 5 sec
Step 2*: 60 °C for 60 sec
Step 3 : 72 °C for 30 sec

Melting Curve: 1 cycle
Step 1 : 95 °C for 50 sec
Step 2 : 37 °C for 50 sec
Step 3**: ramp up to 65 °C

* Fluorescence detection ** Fluorescence detection during 37 - 65 °C ramp with 2 - 4 measurements/°C

For some real-time PCR instruments the probe quencher as well as the usage of a passive reference dye has to be specified. This kit contains probes with a non-fluorescent “dark” quencher and no passive reference dye.
A Color Compensation is necessary for users of the LightCycler 480 System: Color Compensation Set 5 (Product No. KIT230011).
For the Mx3005P instrument: Choose Experiment Type “SYBR® Green (with Dissociation Curve)” and add HEX, ROX, and CY5 channels for data collection in the setup section. Click “Instrument” and “Filter Set Gain Settings” to open the Filter Set Gain Settings dialog box. For FAM, HEX, ROX and Cy5 the Filter Set Gain Setting must be modified to “x1”.

2.4.3 Data Interpretation

Verify results of positive (Control Template) and negative control (H_2O), before interpreting sample results. Always compare samples to positive and negative controls. Review data from each channel and interpret results as described in the tables.

Important note: To use the foodproof Aspergillus Detection LyoKit with the BAX Q7 and BAX®Prep Lysis methods, please use the separately available instructions for the foodproof Aspergillus with BAX®Prep Lysis Kit, KIT2046.

Amplification curves

FAM	HEX	ROX	Cy5	Result Interpretation
+	+	+	+ or -	Positive for *A. flavus, A. terreus,* and *A. niger* and/or *A. fumigatus*
-	+	+	+ or -	Positive for *A. terreus, A. niger* and *A. fumigatus*
+	-	+	+ or -	Positive for *A. flavus, A. niger* and *A. fumigatus*
+	+	-	+ or -	Positive for *A. flavus* and *A. terreus*
-	+	-	+ or -	Positive for *A. terreus*
+	-	-	+ or -	Positive for *A. flavus*
-	-	+	+ or -	Positive for *A. niger* and/or *A. fumigatus*
-	-	-	+	Negative for *A. flavus, A. terreus, A. niger* and *A. fumigatus*
-	-	-	-	Invalid

The Control Template contains all target sequences and usually generates higher fluorescent values than positive samples. This can affect positive/negative calls in automatic analysis of amplification curves by the respective instrument software. Always check results visually for plausibility.

Differentiation of *Aspergillus niger* and *Aspergillus fumigatus*

By melting curve analysis, the amplification product in the ROX channel can be differentiated on a range of PCR instruments: The PCR product of *Aspergillus niger* generates a melt peak at 62 ± 3 °C, whereas the PCR product of *Aspergillus fumigatus* generates a melt peak at 73 ± 3 °C.

2.5 Troubleshooting

Important note: To use the foodproof Aspergillus Detection LyoKit with the BAX Q7 and the BAX®Prep Lysis methods, please use the separately available instructions listed in Section 1.2 (Applicability) for the foodproof Aspergillus with BAX®Prep Lysis Kit (KIT2046).

Problem	Possible Cause	Recommendation
Squashed or crooked tubes, or open / dislodged tube lids after run, or the cycler does not open or close properly.	Wrong tube format.	Choose the correct tube format for your cycler. Tube profile and instrument compatibility chart is available online. If necessary, the samples can be transferred to appropriate PCR vessels after resuspension of the lyophilized PCR mix.
	Wrong placement of tubes.	Place tubes into the cycler in a vertical and balanced order, as described in the instructions for the PCR instrument.
No signal increase is observed, even with positive controls.	Incorrect detection channel has been chosen.	Set channel settings for respective dyes accordingly.
	Pipetting errors.	Check for correct reaction setup and repeat the PCR run. Always run a positive control along with your samples.
	No data acquisition programmed.	Check the cycle programs.
A sample shows no signals, including the internal control. Positive and negative control have proper signals.	Inhibitory effects of the sample material (e.g., caused by insufficient purification).	Use the recommended DNA extraction kit. Dilute samples or pipette a lower amount of sample DNA (e.g., 20 µL PCR-grade water and 5 µL sample instead of 25 µL sample).
Negative control samples are positive.	Carry-over contamination.	Exchange all critical solutions and reagents for DNA/RNA extraction. Repeat the complete experiment with fresh batches of all reagents. Always handle samples, kit components and consumables in accordance with commonly accepted practices to prevent carry-over contamination. Add positive controls after sample and negative control reaction vessels have been sealed.
Fluorescence intensity is too low.	Inappropriate storage of kit components.	Store lyophilized PCR mix at 2 to 8 °C, protected from light and moisture.
	Low initial amount of target DNA.	If possible, increase the amount of sample DNA. Depending on the chosen DNA isolation method, inhibitory effects may occur.

Troubleshooting continues on the next page

Problem	Possible Cause	Recommendation
Strong decrease of fluorescence baseline.	Resuspension of lyophilized PCR mix not complete.	Always resuspend lyophilized PCR mix thoroughly. Use the recommended vortex centrifuge with the correct settings.
Fluorescence intensity varies or changes abruptly during the run.	Insufficient centrifugation of the PCR strips, e.g., resuspended PCR mix is still in the upper part of the vessel or bubbles trapped in the mix.	Always centrifuge PCR strips. Use the centrifuge models and settings recommended in this manual. Avoid the introduction of air bubbles during pipetting.
	Outer surface of the vessel or the seal is dirty (e.g., by direct skin contact).	Always wear gloves when handling the vessels and seal. Do not mark vessels on the outside of the tubes or directly on top of the reaction mix.
Pellets are difficult to dissolve.	The lyophilized PCR mix started to rehydrate.	Store the lyophilized PCR mix always in the aluminum bag with the silica gel pads. Make sure that the lids are tightly closed. Remove strips from the aluminum bag only shortly before PCR setup. Open strip shortly before filling.

2.6 Support

If you have questions or experience any problems with our products, please contact us:

www.hygiena.com/support

Our aim is to provide you with a solution as quickly and effectively as possible. We would also like you to contact us if you have any suggestions for improving the product or in case you would like to use our product for a different application. We highly value your feedback.

3. ADDITIONAL INFORMATION

3.1 Testing Principle

The foodproof kit provides all necessary reagents and a control template for reliable interpretations of results. To ensure maximum reliability of the kit and to prevent misinterpretation of negative results due to inhibition of the amplification, an Internal Control (IC) is included. A hydrolysis probe was designed to bind specifically to the IC, allowing detection in the respective channel, whereas the target DNA is detected in another channel. In case of a negative result due to amplification inhibition of the sample DNA of interest, the amplification of the IC is suppressed as well, whereas a negative result for the sample DNA of interest and amplification of the IC clearly indicates the absence of target DNA in the sample. The real-time PCR kit minimizes contamination risk and contains all reagents (except for template DNA) needed for the detection of target DNA. Primers and probes provide specific detection of target DNA in food and environmental samples, including primary production stage samples. The described performance of the kit is guaranteed for use only on the real-time PCR instruments listed in Section 1.2: Applicability. For other instruments, please contact us.

Step-by-Step Procedure

1. Using the kit's sequence-specific primers in a polymerase chain reaction (PCR), the PCR instrument and the supplied reagents amplify fragments of specific sequences of target DNA.
2. The PCR instrument detects these amplified fragments in real time through fluorescence generated by cleavage of the hybridized probe due to the 5' nuclease activity of the Taq DNA polymerase. The probe is labeled at the 5' end with a reporter fluorophore and at the 3' end with a quencher.
3. During the annealing/elongation phase of each PCR cycle, the probe hybridizes to an internal sequence of the amplicon and is cleaved by the 5' nuclease activity of the Taq DNA polymerase. This cleavage of the probe separates the reporter dye from the quencher dye, increasing the reporter dye signal.
4. The PCR instrument measures the emitted fluorescence of the reporter dye.

Prevention of Carry-Over Contamination

The heat-labile Uracil-DNA N-Glycosylase (UNG) is suitable for preventing carry-over contamination between PCRs. This technique relies on the incorporation of deoxyuridine triphosphate (dUTP) during all amplification reactions, and the pretreatment of all successive PCR mixtures with the heat-labile UNG. The UNG cleaves DNA at any site where a deoxyuridine residue has been incorporated. The resulting abasic sites are hydrolyzed due to the high temperatures during the initial denaturation step, and can no longer serve as PCR templates. The heat-labile UNG is inactivated during the initial denaturation step. Native DNA (e.g., the isolated target genomic DNA) does not contain uracil and is therefore not degraded by this procedure. Since dTTP is replaced with dUTP and UNG is included in this kit, decontamination can be achieved with the provided reagents.

3.2 Trademarks

foodproof®, microproof®, vetproof®, ShortPrep®, StarPrep®, RoboPrep® and LyoKit® are trademarks of Hygiena Diagnostics GmbH.
Hygiena® and BAX® are registered trademarks of Hygiena.
Other brand or product names are trademarks of their respective holders.

3.3 Reference Number

The reference number and original Hygiena Diagnostics GmbH article numbers:
R60272-1 (KIT230145) and R60272-2 (KIT230146).

3.4 Change Index

Version 1, December 2021
First version of the package insert.

Revision A, February 2025:
Rebranding, new document layout and content, new order number.
R 602 72 20 -> INS-KIT230145-46-REVA

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