foodproof®

Aspergillus Detection LyoKit
Ready Reference Guide

Revision A, February 2024

Product No. KIT230145 (LP), KIT230146 (RP), KIT230147 (DP)

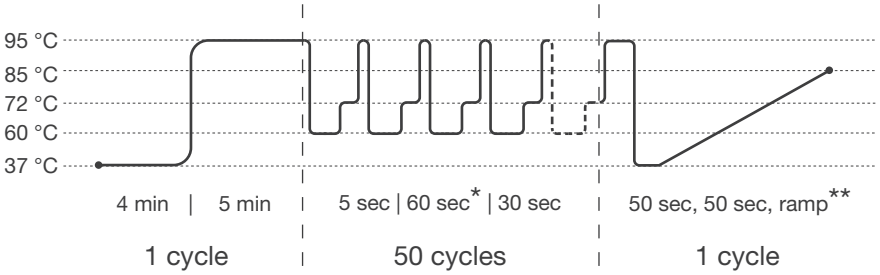
PCR kit for the qualitative detection of *Aspergillus flavus, A. terreus, A. niger* and *A. fumigatus*.

Before starting, it is strongly recommended to read the entire product manual available on our website. For the BAX® Q7 in combination with the BAX Prep *Aspergillus* Lysis Kit (KIT2046), please use the separately available instructions.

PROGRAM SETUP

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).



* Fluorescence detection
** Fluorescence detection during 37 to 85 °C ramp with 1 to 2 measurements/°C

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 85 °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. Color Compensation Set 5 (Product No. KIT230011) is necessary for users of the LightCycler® 480 System. For the Dualo 32 R2 real-time PCR instrument, please open the software, click on 'New', and select the respective template file. Template files can be added by clicking on 'Add' in the 'Select template file' window.

DATA INTERPRETATION

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

	FAM	HEX	ROX	Cy5	Result Interpretation
Amplification Curve	+	-	-	+/-	Positive for *Aspergillus flavus*
	-	+	-	+/-	Positive for *Aspergillus terreus*
	-	-	+	+/-	Positive for *Aspergillus niger* and/or *A. fumigatus*
	-	-	-	+	Negative for all tested *Aspergillus* species
	-	-	-	-	Invalid

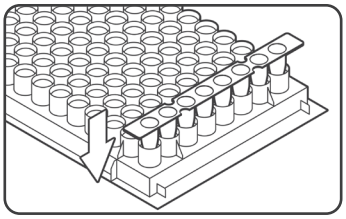
Differentiation:

	Channel	*Aspergillus niger*	*Aspergillus fumigatus*
Melting Curve	ROX	62 ± 3 °C	73 ± 3 °C

RR-KIT230145-46-47-RevA

PREPARATION OF THE PCR MIX

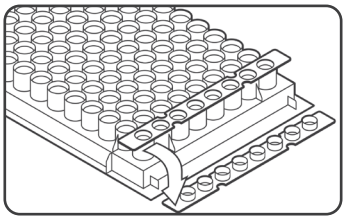
Take appropriate precautions to prevent contamination, e.g., by using filter tips and wearing gloves. Please refer to the Product Instructions for each kit for additional information.



1. PLACE STRIPS IN RACK

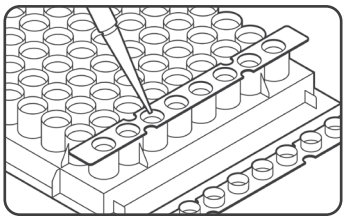
Take needed number of PCR tube strips out of aluminum bag.
Important: close bag tightly afterwards. Place strips in a suitable PCR tube rack.

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



2. DECAP

Carefully open strips immediately before filling and discard caps.
Do not leave open longer than necessary.

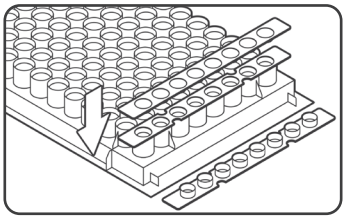


3. ADD SAMPLES AND CONTROLS

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

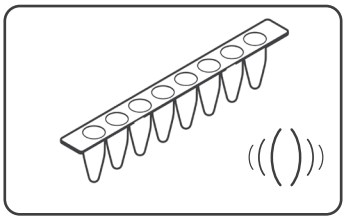
- For extractions using StarPrep® Two Kit: Pipette 20 µL PCR-grade H_2O and 5 µL sample into wells.
- For extractions using BAX Prep *Aspergillus* Lysis Kit: Pipette 30 µL of sample into wells.

(Please see Product Instructions for the respective kits for detailed protocols).



4. SEAL

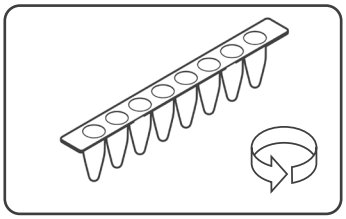
Carefully seal the tubes with the provided 8-cap strips.



5. MIX

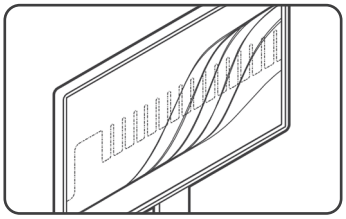
Resuspend pellet after sealing by mixing thoroughly.

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



6. CENTRIFUGE

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



7. START REAL-TIME PCR RUN

Cycle samples as described above.

Place tubes in a vertical, balanced order into the cycler, e.g., two strips can be placed in the first and last column.

foodproof®
Aspergillus Detection LyoKit

KIT230145 /46 /47
Kit for 96 reactions
Store kit at 2 to 8 °C

For food testing purposes
FOR IN VITRO USE ONLY
Made in Germany

Hygiena® | Camarillo, CA 93012 USA | diagnostics.support@hygiena.com | www.hygiena.com