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2-Color *Aspergillus* Detection Kit

Probe based detection kit for *Aspergillus flavus, Aspergillus terreus, Aspergillus niger, and Aspergillus fumigatus.*

RESEARCH USE ONLY

Contents

Product Description

The 2-Color *Aspergillus* Detection Kit is a probe-based kit that allows for the identification of four common species of *Aspergillus* using qPCR. The 2X Master Mix contains multiplexed primers and probes which target the individual species of *Aspergillus*. The probes all report on the FAM channel, giving the user a positive result if any of the species are present.

Species

- *Aspergillus flavus*
- *Aspergillus terreus*
- *Aspergillus niger*
- *Aspergillus fumigatus*

The **2X *Aspergillus* Detection Master Mix** has been tested for up to 20 freeze thaw cycles without a significant loss of activity.

Included in the Kit

- Qty. 2 – 2X *Aspergillus* Detection Master Mix (1.20 mL, 250 rxn total)
- Qty. 1 – *Aspergillus* Positive Control Template (FAM) (80 µL, 40 rxn)
- Qty. 1 – Internal Control Template (HEX/VIC) (500 µL, 250 rxn)
- Qty. 1 – 20X Yellow Dye (1.0 mL)
- Qty. 1 – ROX (0.1 mL)
- Qty. 2 – Water (RNase/DNase/Protease-free) (1.0 mL/tube)

Required But Not Provided

Equipment/Disposables

- qPCR machine and compatible plate(s) or strip tube(s)
- Bench-top centrifuge
- Aerosol filter pipette tips
- Pipettes
- Disposable nitrile gloves
- 1.5 mL microcentrifuge tubes

Storage

- Store all components at - 20 °C

Before First Use

qPCR instruments will generally be classified as being compatible with either a 'high', 'low' or 'no' level of ROX. To get meaningful results, it is therefore critical to ensure that the correct amount of ROX is added to the **2X *Aspergillus* Detection Master Mix** before first use. List of instruments and their ROX levels is provided on page 10 of this manual. If your machine is not listed and you do not know the ROX compatibility of your instrument, please consult your instrument manual.

To reconstitute the Master Mix with the correct level of ROX:

1. Thaw both tubes of **2X *Aspergillus* Detection Master Mix** on ice.
2. Thaw ROX at room temperature (protect from direct light).
3. Depending on the ROX sensitivity of the instrument, add the following amount of ROX and water into each tube of **2X *Aspergillus* Detection Master Mix**:

Instrument ROX Requirement	ROX (µL)	Water (µL)
High Rox	50	0
Low ROX	5	45
No ROX	0	50

4. Mix all components in each **2X *Aspergillus* Detection Master Mix** by pipetting up and down 10 times.

The **2X *Aspergillus* Detection Master Mix** is ready to use.

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Note: The 2-color *Aspergillus* detection qPCR kit is capable of amplifying < 100 copies of *Aspergillus* DNA. Care must therefore be taken to avoid contamination of the kit components with *Aspergillus* DNA template. It is highly recommended to only use aerosol filtered pipette tips under laminar flow during reaction setup. If contamination is an issue, clean the pipettes and lab bench with 3–6 % hypochlorite and use a recently autoclaved or new set of pipette tips for reaction setup.

Note: Ensure all components of the kit are thawed and kept at 4 °C or on ice during reaction setup.

Workflow

*Important: Make sure the **2X Aspergillus Detection Master Mix** has been reconstituted with the correct amount of ROX ("Before First Use", page 4).*

1. Thaw all components on ice. It is necessary to keep all components on ice during reaction setup.
2. Invert each tube 10 times to mix and briefly centrifuge to collect liquid at the bottom of the tube. For tubes containing a low volume of liquid, you may flick the side of the tube 2–3 times, then briefly centrifuge to collect liquid at the bottom of the tube.
3. Add 10 µL of **2X** *Aspergillus* **Detection Master Mix** to a well of a qPCR plate.

Note: qPCR plate with the 2X Aspergillus Detection Master Mix an be kept at room temperature while preparing components in step 4. If a delay is to be expected before proceeding to or during step 4 (Up to 10 minutes), the qPCR plate should be placed at 4 °C.

4. For every test sample, positive and negative control, mix the following components in a 1.5 mL microcentrifuge tube (if performing experiments on technical replicates adjust volumes accordingly):

Component	Volume (10 µL)
20X Yellow Dye	1 µL
DNA Template*	1–6 µL
Internal Control Template	2 µL
Water	up to 10 µL

* For positive control, add 2 µL of Aspergillus positive control template instead of your DNA template. For negative control (no template), do not add any DNA.

5. Ensure components in tube are mixed thoroughly by pipetting or brief vortexing.
6. Briefly centrifuge to collect liquid at the bottom of the tube.
7. Pipette the reaction mixture (10 µL) into wells of a qPCR compatible plate or strip tube containing the **2X *Aspergillus* Detection Master Mix**. The color of the solution should turn green.
8. Briefly centrifuge to collect liquid at the bottom of the well/strip(s).
9. Perform PCR using the following recommended guidelines:

Step	Temperature (°C)	Time	Number of Cycles
Denature	95	2 min	1
qPCR Detection	95	10 sec	35
	60	30 sec	

Interpretation of Results

	Internal Control Ct	Negative Control Ct	Sample Ct	*Aspergillus* Status of Sample
Scenario 1	< 25	–	< 30	POSITIVE
Scenario 2	< 25	–	–	NEGATIVE
Scenario 3	–	–	< 30	POSITIVE
Scenario 4	–	–	–	TEST INVALID

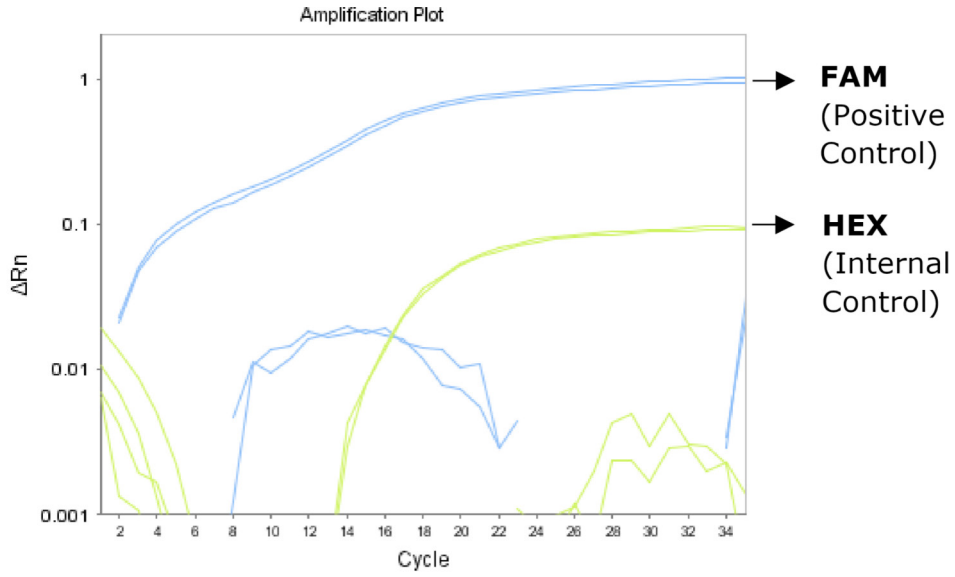
Scenario 1: Amplification occurred in the well with sample (negative control is blank). *Conclusion*: *Aspergillus* DNA detected in sample. *Note*: In cases where a Ct value is observed in the negative control well, the result may still be considered 'positive' if the Ct value of the sample is at least 3 Ct's lower than the negative control.

Scenario 2: Amplification is observed only in the internal control sample. *Conclusion*: *Aspergillus* DNA not detected in sample.

Scenario 3: Same as scenario 1 except no internal control amplification is observed. *Conclusion*: This still constitutes a positive result. The internal control is only informative of a failed qPCR run ***if*** the sample does not amplify.

Scenario 4: If no signal or Ct is observed in the internal control or the sample, the qPCR run is considered unsuccessful. The test is invalid and must be repeated.

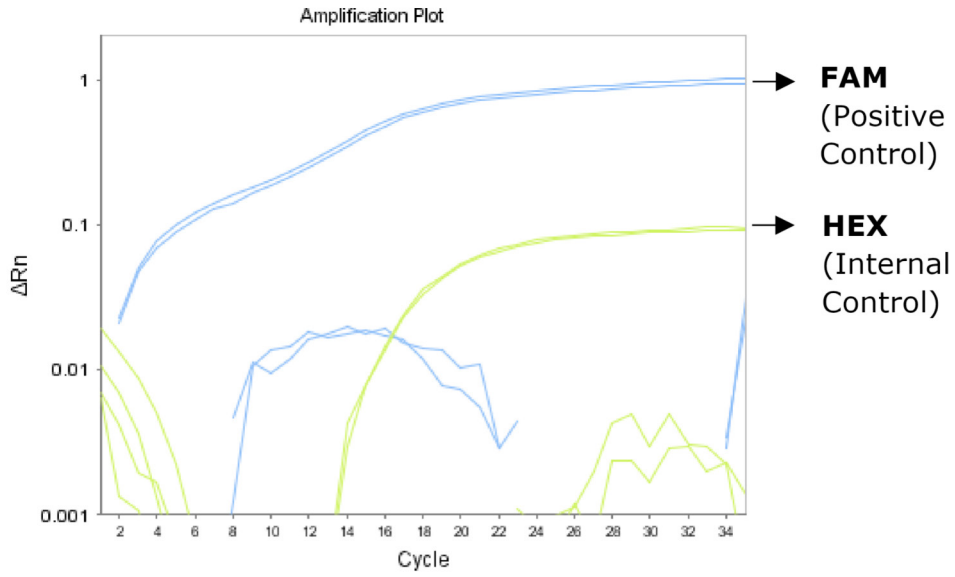
Expected Results



Internal Control Ct	Positive Control Ct	No Template or Negative Control Ct
< 20	< 20	Undetermined or ≥ 34

Table 1. Summary of expected results when using the 2-color *Aspergillus* kit with the internal and positive

Expected Results



Internal Control Ct	Positive Control Ct	No Template or Negative Control Ct
< 20	< 20	Undetermined or ≥ 34

Table 1. Summary of expected results when using the 2-color *Aspergillus* kit with the internal and positive controls included in the kit.

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qPCR Instrument ROX Compatibility Chart

The chart below provides a list of qPCR machines, their manufacturer and ROX compatibilty.

ROX Content	Provider	Real Time PCR Instrument
No ROX (i.e. ROX not recommended)	Biorad	iQ™5, CFX96, CFX384
	Roche	Opticon Lightcycler
	Qiagen	Rotor-Gene™
	Eppendorf	Mastercycler
	Cepheid	SmartCycler
	Antylia Scientific	Eco 48
Low ROX	Biorad	iCycler, MyiQ, MiQ 2, iQ 5, CFX96, CFX384, Chromo4, MJOptiocon, Opticon 2, MiniOpticon
	Cepheid	SmartCycler
	Eppendorf	Mastercycler
	Illumina	Eco Real-Time PCR System
	Qiagen	Rotor-Gene Q, Rotor-Gene 3000, Rotor-Gene 6000
	Roche	LightCycler 480, LightCycler 2.0
	Stratagene	MXP4000P, MX3000P, MX3005P
	ABI	7500, 7500Fast, ViiA 7, QuantStudio™ 3, QuantStudio™ 5, QuantStudio™ 6, QuantStudio™ 7, QuantStudio™ 12K, Flex
ROX or High ROX	ABI	5700, 7000, 7300, 7700, 7900, 7900HT, 7900HTFast, StepOne, StepOnePlus

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