



# Instructions for Use Fungiplex<sup>®</sup> Aspergillus IVD Real-Time PCR Kit

For identification of *Aspergillus* species directly from serum, plasma, and bronchoalveolar lavage

For *in vitro* diagnostic use

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## Document history

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The following table describes important changes from the previous revision of this document.

Section	Changes
-	Change of legal manufacturer to Bruker Daltonics GmbH & Co. KG
-	Option to use an extraction control included.
-	Updates made to instructions for testing on the LightCycler® 480 II and FluoroCycler® XT real-time PCR instruments.

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# 1 Intended use / Intended purpose

The Fungiplex Aspergillus IVD Real-Time PCR Kit (referred to as 'Fungiplex Aspergillus IVD PCR Kit') is a multiplex polymerase chain reaction (PCR) *in vitro* diagnostic (IVD) test for the qualitative detection of *Aspergillus* DNA extracted from serum, plasma, and bronchoalveolar lavage (BAL) fluid obtained from human patients considered at risk of invasive fungal disease (IFD). The test is intended for use by qualified laboratory individuals as an aid to the diagnosis of invasive aspergillosis, specifically of *Aspergillus* spp. and *A. terreus* infections. The results of this test should not be used as the sole basis for diagnosis, treatment or patient management decisions.

## 2 Product description

### Invasive Fungal Disease

Invasive fungal diseases (IFD) are emerging as an increasingly important cause of life-threatening infections and represent a significant cause of morbidity and mortality in patients who receive immunosuppressive therapies, such as those prescribed for solid organ transplants (SOT) and hematopoietic stem cell transplants (HSCT), and in patients suffering immune compromising conditions such as AIDS.

The incidence of human infections caused by fungal species has increased rapidly over the previous two decades as immunocompromised populations increase as a result of improved treatment pathways. Of these infectious species, *Aspergillus* and *Candida* are the most prevalent, accounting for between 70 – 90% of all reported invasive fungal disease depending on patient population<sup>1</sup> (see Table 1).

**Table 1** Proportion of reported invasive fungal infections<sup>1</sup>

	Invasive aspergillosis	Invasive candidiasis	Other
<b>HSCT</b>	43.0%	28.0%	29.0%
<b>SOT</b>	18.8%	52.9%	28.3%
<b>HM</b>	33.0 – 69.0%	13.0 – 44.0%	–
<b>ICU</b>	11.0%	79.0%	10.0%

HSCT – hematopoietic stem cell transplants

SOT – solid organ transplants

HM – hematologic malignancy

ICU – intensive care unit

### *Aspergillus*

*Aspergillus* spp. are opportunistic molds that cause both allergic and invasive syndromes. The genus contains approximately 175 species of which only a minority have been associated with human disease<sup>2</sup>. The prevalence of individual species within the genus is subject to significant variations depending on geographical location and patient population. However, *A. fumigatus* represents the most common cause of human disease followed by *A. flavus*, *A. niger* and *A. terreus*<sup>2,3</sup>. The higher mortality rates associated with *A. terreus*, coupled with its resistance to amphotericin B, a first line treatment for fungal infections, means species level diagnosis is critical for good treatment decisions<sup>4</sup>.

## Diagnosis

The diagnosis of invasive fungal disease can be challenging with difficulties in diagnosis initially leading to considerable variation in how cases were defined. Criteria for identification of IFD for use in clinical trials have been developed by the European Organization for Research and Treatment of Cancer / Mycoses Study Group (EORTC/MSG) allowing improved and consistent definition<sup>5</sup>. Current diagnosis relies upon the presence of risk factors (host characteristics), microbiology, serology (galactomannan (GM) and  $\beta$ -D glucan testing), and various radiological findings<sup>6</sup>. The use of prophylaxis and empiric treatment remains widespread, although the use of biomarkers to guide anti-fungal stewardship is increasing<sup>7</sup>.

Interest in PCR based diagnosis of invasive fungal disease has increased owing to its potential to provide improved speed, sensitivity and specificity over existing methods<sup>8</sup>. A meta-analysis of PCR methods, applied to invasive aspergillosis detection from serum and plasma, indicated that using a single positive PCR gave an overall sensitivity and specificity of 84% and 76% respectively, while a requirement for two positive samples resulted in sensitivity and specificity of 64% and 95%<sup>9</sup>. A meta-analysis on the combined use of PCR and GM in a biomarker surveillance strategy for patients at high risk of IA indicated that a single positive result from either test gave a sensitivity of 99%<sup>10</sup>. When using both PCR and GM, the absence of a positive result provided a negative predictive value of 100%, whereas two positive test results was highly suggestive of active infection and gave a positive predictive value of 88%<sup>10</sup>.

## Fungiplex Aspergillus IVD PCR Kit

The Fungiplex Aspergillus IVD PCR Kit is a multiplex real-time PCR *in vitro* test for the qualitative detection of *Aspergillus* DNA extracted from plasma, serum or bronchoalveolar lavage (BAL) fluid. The assay is designed to be used across common laboratory PCR equipment and can process up to 100 samples from post-extraction to result in less than 2 hours. The species detected, and their respective reporting format, are detailed in Table 2. The rapid, reliable detection provided by this test has the potential to aid earlier diagnosis, while quality controlled manufacture ensures that the user can have full confidence in assay quality and reproducibility.

**Table 2 Fungal pathogens identified using the Fungiplex Aspergillus IVD PCR Kit**

Reported target	Species detected
<i>Aspergillus</i> spp.	<i>A. fumigatus</i>
	<i>A. flavus</i>
	<i>A. niger</i>
<i>A. terreus</i>	<i>A. terreus</i>

## References

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2. M. Richardson, C. Lass-Flörl, *Clin. Microbiol. Infect.*, **2008**, 14, 15-24
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4. M. A. Pfaller, P. G. Pappas, J. R. Wingard, *Clin. Infect. Dis.*, **2006**, 43, Suppl. 1: S3-S14
5. B. De Pauw *et al.* EORTC/MSG Cooperative group, *Clin. Infect. Dis.*, **2008**, 46, 1813-1821
6. T. F. Patterson *et al.*, *Clin. Infect. Dis.*, **2016**, 63, e1-e60
7. J. A. Maertens *et al.*, *J. Antimicrob. Chemother.*, **2016**, 71, Suppl 2: ii23–ii29

8. T. K. Kourkoumpetis *et al.*, *Clin. Infect. Dis.*, **2012**, 54, 1322–1331
9. M. Aravinitis *et al.*, *J. Clin. Microbiol.*, **2014**, 52, 3731–3742
10. M. Aravinitis *et al.*, *Clin. Infect. Dis.*, **2015**, 61, 1263-72

## 2.1 Fungiplex Aspergillus IVD Real-Time PCR Kit components

Reagents are provided in quantities that will allow 100 tests to be performed.

### Fungiplex Aspergillus Real-Time PCR Kit

Reagent	Volume per tube	Number of tubes	Cap color	Reactions per tube
PCR Master Mix	1.2 mL	1	Orange	100
Aspergillus Master Mix	120 µL	1	Purple	100
PCR Water	800 µL	1	White	100

### Fungiplex Aspergillus Control Kit

Reagent	Volume per tube	Number of tubes	Cap color
Ext / PCR Control	650 µL	2	Red
Positive Control Aspergillus	60 µL	3	Green
Negative Control	240 µL	3	White

## 2.2 Ordering information


Product	Part number
Fungiplex Aspergillus IVD PCR Kit	1849974
Colour Compensation Kit 1	1880885

## 3 Inspection, storage and stability

### 3.1 Inspection on arrival

Check the Fungiplex Aspergillus IVD PCR Kit on arrival. If the packaging is damaged the kit must not be used. Additionally, all reagents contained within the Fungiplex Aspergillus IVD PCR Kit are shipped frozen and should arrive frozen. If reagents are not frozen upon receipt or if the tubes have been compromised during shipment, contact Bruker Daltonics GmbH & Co. KG for assistance.

### 3.2 Storage temperature

 <p>-25°C      -15°C</p>	<p>The Fungiplex Aspergillus IVD PCR Kit is shipped on dry ice and should be stored within this advised temperature range immediately on receipt.</p>
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### 3.3 Suitable storage area

The internal reagent box named 'Fungiplex Aspergillus Real-Time PCR Kit' should be stored in an **amplicon free laboratory**.

The internal reagent box named 'Fungiplex Aspergillus Control Kit', which includes all necessary controls, should be stored in the area of the facility where the **DNA template / sample template is added**.

### 3.4 Stability

The Fungiplex Aspergillus IVD PCR Kit has demonstrated that the performance is not affected when used over 10 freeze-thaw cycles.

All reagents contained within the Fungiplex Aspergillus IVD PCR Kit have been validated for use within the expiration date stated on the kit label and must be used within this time period.



## 4 Risk and safety information

### 4.1 General precautions

- The Fungiplex Aspergillus IVD PCR Kit is for *in vitro* diagnostic use.
- This test is only for use with the PCR platforms on which the process has been validated.
- A trained professional should interpret results generated from the Fungiplex Aspergillus IVD PCR Kit in conjunction with other diagnostic tests and clinical presentation.
- Test procedure should be performed as outlined within this Instruction for Use. Any deviation from the outlined protocols may result in assay failure or generate erroneous results.
- This test is for use with DNA extracted from plasma, serum and bronchoalveolar lavage (BAL).
- Standard precautions and institutional guidelines should be followed when handling all samples.
- Low concentrations of DNA can be unstable if stored for long periods. Bruker advises that sample storage time should be minimized before testing.
- Do not use reagents after their stated expiry date.
- Do not mix reagents from different tubes or kits, even if they are from the same lot.
- Do not substitute reagents from different manufacturers.
- Take care to preserve the purity of kit reagents. Avoid contamination from positive controls and specimens by following good laboratory practice and organizing and segregating workflow appropriately.
- Ensure all additional required consumables are DNase / RNase free.
- Wear protective clothing and disposable gloves when handling kit reagents.
- Do not use kit reagents if the label seal is broken upon receipt.
- Do not eat, drink or smoke in areas where specimens or kit reagents are being handled.
- Dispose of unused reagents and waste in accordance with country, state and local regulations.

### 4.2 Chemical risks

There are no hazardous substances included in the manufacture of the Fungiplex Aspergillus IVD PCR Kit. The composition of all kit reagents presents no specific risk to the user or to their property.

Additional chemicals and materials may be required for procedures described in these Instructions for Use. Carefully read any warnings, instructions, or Safety Data Sheets provided by the supplier and follow general safety regulations when handling chemicals, biohazards, or other materials.

### 4.3 Biological risks

The Fungiplex Aspergillus IVD PCR Kit involves working with potentially dangerous and transmissible biological material. Personnel working with these materials are responsible for reading and following all necessary health and safety precautions.

It is very important to wear appropriate personal protective equipment at all times; a lab coat, protective gloves and safety glasses as a minimum.

## 4.4 Limitations of the procedure

- This assay is a qualitative test and does not provide quantitative data on any detected organism.
- False positive results may arise from external contamination of original sample or contamination during PCR set-up.
- All results generated by this assay should be interpreted by a trained healthcare professional in conjunction with other diagnostic tests and clinical presentation.
- Positive results do not rule out co-infection with additional organisms that are not detected via this test.
- Extraction efficiency may impact results and could result in false negative outcomes.
- Preliminary data indicates that stability of extracted DNA may be affected by freezing and storage. It is therefore recommended that samples are processed as quickly as possible after collection.
- A positive result for *Aspergillus* spp. is possible if the infecting agent is *Penicillium* spp.

## 5 Materials required but not provided

### 5.1 General equipment

#### Equipment

- Recommended nucleic acid extraction platform
- Validated real-time thermal cycler instrument
- Class II hood / PCR station
- Vortex mixer
- Mini centrifuge
- Centrifuge capable of microplate processing (recommended)
- Fridge (2 to 8°C) and freezers (-25°C to -15°C)
- Micropipettes for volumes of 1 to 1000 µL

#### Reagents

- Extraction reagents
- Negative extraction control (recommended, for example nuclease free water)

#### Consumables

- Appropriate DNase / RNase free plasticware for PCR preparation
- DNase / RNase free pipette tips
- Disposable gloves, powderless
- PCR plasticware compatible with the thermal cycler of choice

### 5.2 Real-time PCR instrumentation

The Fungiplex Aspergillus IVD PCR Kit has been validated across multiple real-time PCR instruments. The validated instruments and a list of consumables used throughout verification and validation studies are advised below in Table 3.

**Table 3 Real-time PCR instrumentation specific details**

Real-time PCR instrumentation manufacturer	Real-time PCR platform model	Real-time PCR instrument software version	Validated consumables
Applied Biosystems	ABI® 7500 Fast	2.3	MicroAmp™ Fast Optical 96-Well Reaction Plates MicroAmp™ Optical 8-Cap Strips

Real-time PCR instrumentation manufacturer	Real-time PCR platform model	Real-time PCR instrument software version	Validated consumables
Bio-Rad	CFX96™	CFX Manager 3.1	MicroAmp™ Fast Optical 96-Well Reaction Plates MicroAmp™ Optical 8-Cap Strips
		CFX Manager IVD Edition 1.6	Bio-Rad, Hard-Shell® 96- Well PCR Plate, low-profile, skirted, white Bio-Rad, Optical Flat 8-Cap Strips <b>Note</b> Bio-Rad White 8-Tube PCR Strips are not suitable for use with the Fungiplex Aspergillus Assay.
Qiagen	Rotor- Gene Q 5plex HRM	2.3.1 OR Q-Rex 1.0.0	Qiagen Strip Tubes and Caps, 0.1 mL
Roche	LightCycler® 480 II	1.5.1	4titude® FrameStar 480/90 PCR plates <sup>1</sup> LightCycler® plate seal LightCycler® 480 Multiwell Plate 96 (white) MicroAmp™ Optical 8-Cap Strips
Applied Biosystems	QuantStudio™ 5	1.3.1	MicroAmp™ Fast Optical 96-Well Reaction Plates MicroAmp™ Optical 8-Cap Strips
Applied Biosystems	StepOnePlus	2.2.2	MicroAmp™ Fast Optical 96-Well Reaction Plates MicroAmp™ Optical 8-Cap Strips
Bio Molecular Systems	Mic qPCR Cycler	2.6.4	Mic tubes and caps supplied by Bio Molecular Systems
Hain Lifescience	FluoroCycler® XT	IVD v 1.0.1.4.20.62	4titude® FrameStar 480/90 PCR plates 4titude® Clear Weld Seal Mark II seals

<sup>1</sup> Some variability in *A. terreus* detection has been observed when using the Roche LightCycler® 480 Multiwell Plate 96 (white) and therefore the 4titude® FrameStar 480/90 PCR plates are recommended

## 6 Specimen collection and DNA extraction

The sensitivity achieved by real-time PCR assays is inherently linked to the quality of the extracted DNA and therefore samples must be collected and stored appropriately following the guidelines below.

- Samples should be extracted from plasma, serum or bronchoalveolar lavage on available extraction platforms following the recommendations for extracting DNA from *Aspergillus fumigatus* published by the European *Aspergillus* PCR Initiative<sup>11,12</sup> (EAPCRI).
- Good laboratory practice recommends including at least one positive and negative extraction control per analysis.
- Extracted samples should be stored between –80°C and –20°C for long term storage.

**Note** Low concentrations of DNA can be unstable if stored for long periods.

### References

11. P. L. White *et al.*, *J. Clin. Microbiol.*, **2010**, 3753-5
12. P.L. White *et al.*, *J. Clin. Microbiol.*, **2011**, 3842-8

### 6.1 Recommended extraction protocols used throughout validation

The protocols used for the extraction of DNA throughout the validation studies are referenced in Table 4. Bruker has the ability to recommend these extraction procedures but accepts no responsibility for the performance of any of the stated protocols and advises each user to perform an internal validation on their specific platform / procedure.

For extraction from BAL samples, bead beating steps are recommended.

1. BAL samples should be stored at -80°C until used.
2. Centrifuge the sample at 10,000 g for 10 minutes.
3. Remove supernatant and retain until step 5.
4. Add approximately 10 beads to the sample and disrupt the pellet with the beads using a vortex or bead beater for 30 seconds.
5. Pulse centrifuge and add up to 500 µL of the corresponding retained supernatant.
6. Use the supernatant to wash the disrupted pellet and beads.
7. Transfer the supernatant into a fresh tube and use with an extraction platform.
8. Elute DNA in < 100 µL.

**Table 4**      **Extraction protocols**

<b>Extraction platform</b>	<b>Extraction kit used</b>	<b>Extraction input volume</b>	<b>Extraction elution volume</b>
<b>Qiagen QIA Symphony</b>	DSP virus pathogen Midi Kit 1000	1000 µL	65 µL
<b>Qiagen EZ1 Advanced XL</b>	EZ1 DSP Virus kit	400 µL	60 µL
<b>bioMérieux NucliSENS® easyMag®</b>	Generic 2.0.1 with Nuclisens lysis buffer	500 µL	60 µL
<b>bioMérieux eMAG®</b>	Generic 3.0.4 with off-board lysis	500 µL	60 µL
<b>MANUAL extraction procedure</b>	QIAamp UltraSens Virus Kit	35 µL	32 µL
<b>Hain GXT-12</b>	Nucleic Acid Kit	550 µL	100 µL

## 7 Test procedure

The control material (**Ext / PCR Control**) contained within the Fungiplex Aspergillus IVD PCR Kit can be used as either an extraction control or a PCR inhibition control.



### ATTENTION

Bruker recommends the use of a clean room (PCRsuite) to set up all PCR reactions with addition of template in a suitable area out with the clean room facility. To avoid any risk of contamination, users should follow good laboratory practice at all times and segregate workflow appropriately.

**DNase / RNase free plasticware should be used.**

- ▶ If **Ext / PCR Control** is to be used as an **extraction control**, proceed to section 7.1. For this procedure the control material should be added prior to extraction and NOT included in the **PCR Master Mix**.
- ▶ If **Ext / PCR Control** is to be used as a **PCR inhibition control**, proceed to section 7.2. For this procedure the control material should be added to the **PCR Master Mix** after extraction.

### 7.1 Use of Ext / PCR Control as an extraction control

#### 7.1.1 Addition of extraction control to sample

- Remove the vial Ext / PCR Control (contained within the Fungiplex Aspergillus Control Kit) from the freezer and allow to thaw.
- Add 10 µL of Ext / PCR Control to each sample prior to extraction.
- Ensure at least one negative sample is included in the extraction run – see section 7.1.2.
- Extract the samples following existing internal procedures on a commercial extraction platform following guidelines recommended in section 6.

#### 7.1.2 PCR set-up when using Ext / PCR Control as an extraction control

- Before beginning PCR set-up, the user has the responsibility to assign samples to the appropriate wells within the appropriate plasticware; this information should be recorded for traceability purposes.
- Within an amplicon free area, remove the Fungiplex Aspergillus IVD PCR Kit from the freezer and allow the reagents to thaw.
- Briefly vortex and centrifuge the supplied reagents.
- Prepare a PCR reaction mastermix using the reagents and volumes detailed in Table 5.
- Volumes are given per reaction well and should be multiplied by the number of reaction wells, taking into account the number of samples, positive and negative controls in the analysis. The prepared PCR reaction mastermix should be thoroughly mixed and centrifuged for 10 seconds.

**Table 5** Preparation volumes for the PCR reaction mastermix in an amplicon free area

Assay kit reagents	PCR reaction mastermix
PCR Master Mix	10.0 µL
Aspergillus Master Mix	1.0 µL
PCR Water	4.0 µL
<b>Total volume (per well)</b>	<b>15.0 µL</b>

**Example:** For 10 reaction wells (8 samples, 1 positive control and 1 negative control), the total volume of the PCR reaction mastermix will be  $10 \times 15 = 150 \mu\text{L}$ .

- Before dispensing into reaction wells, transfer the PCR reaction mastermix to the template / sample addition area and dispense 15 µL of the PCR reaction mastermix into the relevant tubes / wells of the appropriate plasticware.
- Remove the Fungiplex Aspergillus Control Kit from the freezer and allow the reagents to thaw. Briefly vortex and centrifuge the reagents.
- For each sample, add 5 µL of extracted sample material to the PCR reaction mastermix in the required well (see Table 6).
- For each positive control sample, add 5 µL of Positive Control Aspergillus to the PCR reaction mastermix in the required well. At least one positive control reaction should be included to provide details on reaction efficiency.

**Note** The Positive Control Aspergillus should be added directly to the PCR mastermix and should not undergo any extraction procedures.

- For each negative control sample, add 5 µL of negative extracted material to the PCR reaction mastermix in the required well. At least one negative control reaction should be included to provide details on contamination.

**Note** This is NOT the Negative Control supplied by Bruker.

- For a negative control sample, add 1 µL **Ext / PCR Control** and 4 µL of **Aspergillus Negative Control** to the PCR reaction mastermix in the required well. At least one negative control reaction should be included to provide details on contamination.

**Table 6** Dispensed volumes of final PCR reaction mastermix per reaction well

PCR reaction well composition	Final PCR reaction mastermix
PCR reaction mastermix (from Table 5)	15.0 µL
Sample / Control material template	5.0 µL
<b>Final volume (per well)</b>	<b>20.0 µL</b>

- The plasticware should then be sealed and transferred to a validated thermal cycler for amplification.

**Note** Ensure the samples are centrifuged briefly before placing in the thermal cycler.

**Note** Please refer to the instrument manual for instructions on setting up an amplification run. Amplification should be carried out according to the instrument-specific parameters detailed in section 8 of this Instructions for Use.



## 7.2 PCR set-up when using Ext / PCR Control as a PCR inhibition control

- Before beginning PCR set-up, the user has the responsibility to assign samples to the appropriate wells within the appropriate plasticware; this information should be recorded for traceability purposes.
- Within an amplicon free area, remove the Fungiplex Aspergillus IVD PCR Kit from the freezer and allow the reagents to thaw.
- Briefly vortex and centrifuge the supplied reagents.
- Prepare a PCR reaction mastermix using the reagents and volumes detailed in Table 7.
- Volumes are given per reaction well and should be multiplied by the number of reaction wells, taking into account the number of samples, positive and negative controls in the analysis. The prepared PCR reaction mastermix should be thoroughly mixed and centrifuged for 10 seconds.

**Table 7** Preparation volumes for the PCR reaction mastermix

Assay kit reagents	PCR reaction mastermix
PCR Master Mix	10.0 µL
Aspergillus Master Mix	1.0 µL
PCR Water	3.0 µL
<b>Total volume (per well)</b>	<b>14.0 µL</b>

**Example:** For 10 reaction wells (8 samples, 1 positive control and 1 negative control), the final volume of the PCR reaction mastermix will be  $10 \times 14 = 140 \mu\text{L}$ .

- Before dispensing into reaction wells, transfer the PCR reaction mastermix to the template / sample addition area for inclusion of the Ext / PCR Control.
- Remove the Fungiplex Aspergillus Control Kit from the freezer and allow the reagents to thaw. Briefly vortex and centrifuge the reagents.
- The Ext / PCR Control material should be added to the bulk PCR reaction mastermix. The Ext / PCR Control is added at 1 µL per reaction well (see Table 8).

**Table 8** Preparation volumes for the PCR reaction mastermix with inclusion of Ext / PCR Control

Mastermix reagents + Ext / PCR Control	PCR reaction mastermix
PCR reaction mastermix (from Table 7)	14.0 µL
Ext / PCR Control	1.0 µL
<b>Total volume (per well)</b>	<b>15.0 µL</b>

**Example:** For 10 reaction wells (8 samples, 1 positive control and 1 negative control) 10 µL of the Ext / PCR Control material must be added to the bulk mastermix. For 10 reaction wells this will provide a total PCR reaction mastermix volume of 150 µL.

- Dispense 15 µL of PCR reaction mastermix prepared in Table 8 into appropriate plasticware.
- For each sample, add 5 µL of extracted sample material to the PCR reaction mastermix (see Table 9).

- For each positive control, add 5 µL of Positive Control Aspergillus to the PCR reaction mastermix. At least one positive control reaction should be included to provide details on reaction efficiency.
- For each negative control, add 5 µL of Negative Control to the PCR reaction mastermix. At least one negative control reaction should be included to provide details on contamination.

**Table 9**      **Dispensed volumes of final PCR reaction mastermix per reaction well**

PCR reaction well composition	Final PCR reaction mastermix
PCR reaction mastermix + Ext / PCR Control (from Table 8)	15.0 µL
Sample / Control material template	5.0 µL
<b>Final volume (per well)</b>	<b>20.0 µL</b>

- The plasticware should then be sealed and transferred to a validated thermal cycler for amplification.

**Note**      Ensure the samples are centrifuged briefly before placing in the thermal cycler.

**Note**      Please refer to the instrument manual for instructions on setting up an amplification run. Amplification should be carried out according to the instrument-specific parameters detailed in section 8 of this Instructions for Use.

## 8 Thermal cycler conditions

### 8.1 ABI® 7500

Refer to the instruction manual for information on how to operate the real-time PCR instrument and perform data analysis.

This instrument collects all dyes as default – see Table 10 for the dyes used with the Fungiplex Aspergillus IVD PCR Kit.

**Table 10** Detector channels used on the ABI® 7500 instrument

	GREEN	YELLOW	ORANGE
<b>Reporter Dye Channel</b>	FAM	JOE	ROX
<b>Quencher</b>	None	None	None
<b>Target detected</b>	Ext / PCR Control	<i>Aspergillus</i> spp.	<i>A. terreus</i>

Configure the ABI® 7500 with the following settings:

- **Instrument type: 7500 Fast (96 wells)**
- **Experiment type: Quantitation – Standard Curve**
- **Reagents used: TaqMan® Reagents**
- **Ramp speed: Standard**
- **Reaction volume: 20 µL**
- **Passive reference dye: None**

See Table 11 for the thermal profile settings. Data should be collected for all 45 cycles.

**Table 11** PCR parameters for the Fungiplex Aspergillus IVD PCR Kit

Step	Temperature (°C)	Time	Number of cycles	Data collection
<b>Activation</b>	95	15 minutes	1	Off
<b>Denaturation</b>	95	5 seconds	45	Off
<b>Annealing / extension</b>	60	30 seconds		On

To analyze the data, ensure the **Passive reference dye** is set to **None** and in **Options** deselect the box for **Auto threshold** for each target. The **Baseline** should continue to be calculated **Automatically**.

Use the information in Table 12 to set the **Threshold** for each detector channel in the **Analysis settings** window.

**Table 12** ABI® 7500 threshold values for the Fungiplex Aspergillus IVD PCR Kit

Channel	FAM	JOE	ROX
<b>Target(s)</b>	Ext / PCR Control	<i>Aspergillus</i> spp.	<i>A. terreus</i>
<b>Threshold</b>	10000	10000	15000

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It is important to visually inspect the amplification plots for each sample to ensure that the results recorded are due to true amplification and cannot be attributed to background noise recorded above the defined thresholds.

## 8.2 Rotor-Gene Q

Refer to the instruction manual for information on how to operate the real-time PCR instrument and perform data analysis.

Select the appropriate fluorophore within in each channel and assign to the relevant target, as shown in Table 13.

**Table 13** Detector channels used on the Rotor-Gene Q instrument

Reporter Dye Channel	GREEN	YELLOW	ORANGE
Target detected	Ext / PCR Control	<i>Aspergillus</i> spp.	<i>A. terreus</i>

Configure the Rotor-Gene Q / Q-Rex software with the following settings:

- Select **72-well rotor**
- **Reaction volume: 20 µL**
- **Auto Gain** should be used for the selected dye channels only. For Rotor-Gene Q set to implement before first acquisition on the default well, for Q-Rex use the **Default / Auto-gain** method.

See Table 14 for the thermal profile settings. Data should be collected for all 45 cycles.

**Table 14** PCR parameters for the Fungiplex Aspergillus IVD PCR Kit

Step	Temperature (°C)	Time	Number of cycles	Data collection
<b>Activation</b>	95	15 minutes	1	Off
<b>Denaturation</b>	95	5 seconds	45	Off
<b>Annealing / extension</b>	60	30 seconds		On

To analyze data apply the following settings:

### Rotor-Gene Q

- **Normalization:** select **Dynamic Tube** and **Slope Correct**.
- Analyze all cycles and enter the **Threshold** and **Outlier removal** values for each channel as detailed in Table 15.

### Rotor-Gene Q-Rex

- Use the **Target Detection Analysis** method.
- **Filter data:** select **Light** for **Degree of data smoothing**, **Remove non-amplified curves with** and enter the **Fluorescence change percentage** for each channel as detailed in Table 15 (labeled as '**Outlier removal**').
- **Normalization:** select **Dynamic Tube** and **Use Noise Slope Correction**.
- Analyze all cycles and enter the **Threshold** for each channel as detailed in Table 15.

**Table 15 Rotor-Gene Q and Q-Rex threshold values for the Fungiplex Aspergillus IVD PCR Kit**

<b>Channel</b>	<b>GREEN</b>	<b>YELLOW</b>	<b>ORANGE</b>
<b>Target(s)</b>	Ext / PCR Control	<i>Aspergillus</i> spp.	<i>A. terreus</i>
<b>Threshold</b>	0.100	0.126	0.040
<b>Outlier removal</b>	3%	3%	10%

It is important to visually inspect the amplification plots for each sample to ensure that the results recorded are due to true amplification and cannot be attributed to background noise recorded above the defined thresholds.

## 8.3 CFX96™

Refer to the instruction manual for information on how to operate the real-time PCR instrument and perform data analysis.

This instrument collects all dyes as default – see Table 16 for the dyes used with the Fungiplex Aspergillus IVD PCR Kit.

**Table 16** Detector channels used on the CFX96™ instrument

	GREEN	YELLOW	ORANGE
<b>Reporter Dye Channel</b>	FAM	VIC	ROX
<b>Target detected</b>	Ext / PCR Control	<i>Aspergillus</i> spp	<i>A. terreus</i>

Configure the CFX96™ with the following settings

- **Sample volume:** 20 µL
- **Scan Mode:** All channels
- **Plate Type:** BR Clear / BR White (dependent on plasticware used)

**Note** Fungiplex Aspergillus IVD PCR Kit is not compatible with Bio-Rad White 8-Tube PCR Strips.

See Table 17 for the thermal profile settings. Data should be collected for all 45 cycles.

**Table 17** PCR parameters for the Fungiplex Aspergillus IVD PCR Kit

Step	Temperature (°C)	Time	Number of cycles	Data collection
<b>Activation</b>	95	15 minutes	1	Off
<b>Denaturation</b>	95	5 seconds	45	Off
<b>Annealing / extension</b>	60	30 seconds		On

To analyze the data, under **Baseline Setting** select the following:

- **Baseline Subtracted Curve fit**
- **Apply fluorescent drift correction** (not applicable when using the IVD software)

To set the threshold values for each detector channel navigate to **Settings > Baseline threshold > Single threshold > User defined** and enter the values from Table 16. **Baseline** should remain **Auto-calculated**.

**Table 18** CFX96™ threshold values for the Fungiplex Aspergillus IVD PCR Kit

Channel	FAM	VIC	ROX
<b>Target(s)</b>	Ext / PCR Control	<i>Aspergillus</i> spp	<i>A. terreus</i>
<b>Threshold</b>	50	50	50

It is important to visually inspect the amplification plots for each sample to ensure that the results recorded are due to true amplification and cannot be attributed to background noise recorded above the defined thresholds.

## 8.4 QuantStudio™ 5

Refer to the instruction manual for information on how to operate the real-time PCR instrument and perform data analysis.

This instrument collects all dyes as default – see Table 19 for the dyes used with the Fungiplex Aspergillus IVD PCR Kit.

**Table 19** Detector channels used on the QuantStudio™ 5 instrument

	GREEN	YELLOW	ORANGE
<b>Reporter Dye Channel</b>	FAM	VIC	ROX
<b>Quencher</b>	None	None	None
<b>Target detected</b>	Ext / PCR Control	<i>Aspergillus spp.</i>	<i>A. terreus</i>

Configure the QuantStudio™ 5 with the following settings:

- **Sample volume: 20 µL**
- **Standard mode** (with ramp rates of 1.6°C/second)

See Table 20 for the thermal profile settings. Data should be collected for all 45 cycles.

**Table 20** PCR parameters for the Fungiplex Aspergillus IVD PCR Kit

Step	Temperature (°C)	Time	Number of cycles	Data collection
<b>Activation</b>	95	15 minutes	1	Off
<b>Denaturation</b>	95	5 seconds	45	Off
<b>Annealing / extension</b>	60	30 seconds		On

To analyze the data, ensure the **Passive reference dye** is set to **None** and in **Options** deselect the box for **Auto threshold** for each target. The **Baseline** should continue to be calculated **Automatically**.

Use the information in Table 21 to set the threshold values for each detector channel in the **Analysis settings** window.

**Table 21** QuantStudio™ 5 threshold values for the Fungiplex Aspergillus IVD PCR Kit

Channel	FAM	VIC	JUN
<b>Target(s)</b>	Ext / PCR Control	<i>Aspergillus spp.</i>	<i>A. terreus</i>
<b>Threshold</b>	4200	4200	23000

It is important to visually inspect the amplification plots for each sample to ensure that the results recorded are due to true amplification and cannot be attributed to background noise recorded above the defined thresholds.



## 8.5 LightCycler® 480 II

On request, the Fungiplex Aspergillus IVD PCR Kit is supplied with a Colour Compensation Kit 1 for use with the LightCycler® 480 II. If you have not received this kit or if you want to repeat this procedure, please contact [care@bruker.com](mailto:care@bruker.com)

Follow the instructions in IFU Colour Compensation Kit 1 (1880885) to create a color compensation file before running any samples with the Fungiplex Aspergillus IVD PCR Kit.

**Note** Prior to testing of the Fungiplex Aspergillus IVD real-time PCR assay on the LC480 II instrument, a colour compensation file must be generated using the accessory **Colour Compensation Kit 1**. Follow the instructions stated in the IFU of Colour Compensation Kit 1 to generate a 'CC Kit 1' colour compensation file. Follow the instructions below to generate an Aspergillus assay specific CC file.

In the file generated by CC Kit 1, select the **Subset Editor** tab, click **+** and highlight all the wells in use, name (suggested subset name: *Fungiplex Aspergillus*) and click **Apply**.

Go to sample editor:

- Ensure **Color Comp** is selected in **Step 1: Select Workflow**.
- In **Step 2: Select Samples**, highlight all replicate wells from each individually prepared mastermix.
- In **Step 3: Edit Color Comp Properties**, assign each mastermix to its target name. These are provided in Table 22. Click **Make Replicates**.

Go to the **Analysis** tab and select **Color compensation**.

Under the **Subset** drop down menu select the file name previously entered. Click **Calculate**.

Click **Save** and store in an appropriate location. Then click on **Save CC** object at the bottom right of the screen, select the **Special Data** folder > select the **CCC** folder and name appropriately (suggested object name: *CC Fungiplex Aspergillus*).

**Table 22** Dominant channels associated with *Aspergillus* targets for creation of color compensation files

Samle name	Dominant channel
Ext / PCR Control	Channel 1
<i>Aspergillus</i> spp.	Channel 2
<i>A. terreus</i>	Channel 3
Blank	Water

Use this Aspergillus specific color compensation file to perform data analysis on resultant Fungiplex Aspergillus Assay data.

Refer to the instruction manual for information on how to operate the real-time PCR instrument and perform data analysis.

Table 23 details the targets detected by the corresponding filter combinations. Set these dyes as the reporter dyes.

**Table 23** Detector channels used on the LightCycler® 480 II instrument

	Channel 1	Channel 2	Channel 3
<b>Filter Comb</b>	465 – 510	533 - 610	618 – 660
<b>Target detected</b>	Ext / PCR Control	<i>Aspergillus</i> spp.	<i>A. terreus</i>

Use the detection format set up in during Colour Compensation Kit 1 testing (IFU 1880885) (suggested file name: CC Kit 1) and use the LightCycler® 480 II with the following settings:

- **Reaction volume: 20 µL**

See Table 24 for the thermal profile settings. Data should be collected for all 45 cycles.

**Table 24** PCR parameters for the Fungiplex Aspergillus IVD PCR Kit

Step	Temperature (°C)	Time	Number of cycles	Data collection
<b>Activation</b>	95	15 minutes	1	None
<b>Denaturation</b>	95	5 seconds	45	None
<b>Annealing / extension</b>	60	30 seconds		Acquire

Analyze data with **Absolute quantification / second derivative max**. Data is analyzed using the color compensation file prepared as above. The use of thresholds is not applicable – see Table 25.

**Table 25** LightCycler® 480 II threshold values for the Fungiplex Aspergillus IVD PCR Kit

Channel	Channel 1	Channel 2	Channel 3
<b>Target(s)</b>	Ext / PCR Control	<i>Aspergillus</i> spp.	<i>A. terreus</i>
<b>Threshold</b>	2nd derivative auto-calculated	2nd derivative auto-calculated	2nd derivative auto-calculated

It is important to visually inspect the amplification plots for each sample to ensure that the results recorded are due to true amplification and cannot be attributed to background noise recorded above the defined thresholds.

## 8.6 StepOnePlus

Refer to the instruction manual for information on how to operate the real-time PCR instrument and perform data analysis.

Select the appropriate fluorophore within in each channel and assign to the relevant target, as shown in Table 26.

**Table 26** Detector channels used on the StepOnePlus instrument

	GREEN	YELLOW	ORANGE
<b>Reporter Dye Channel</b>	FAM	JOE	ROX
<b>Quencher</b>	None	None	None
<b>Target detected</b>	Ext / PCR Control	<i>Aspergillus</i> spp.	<i>A. terreus</i>

Configure the StepOnePlus with the following settings:

- **Instrument type: StepOnePlus (96 wells)**
- **Experiment type: Quantitation**
- **Quantitation Method: Std Curve**
- **Reagents used: TaqMan® Reagents**
- **Ramp speed: Standard**
- **Reaction volume: 20 µL**
- **Passive reference dye: None**

See Table 27 for the thermal profile settings. Data should be collected for all 45 cycles.

**Table 27** PCR parameters for the Fungiplex Aspergillus IVD PCR Kit

Step	Temperature (°C)	Time	Number of cycles	Data collection
<b>Activation</b>	95	15 minutes	1	Off
<b>Denaturation</b>	95	5 seconds	45	Off
<b>Annealing / extension</b>	60	30 seconds		On

To analyze the data, ensure the **Passive reference dye** is set to **None** and in **Options** deselect the box for **Auto threshold** for each target. The **Baseline** should continue to be calculated **Automatically**.

Use the information in Table 28 to set the **Threshold** for each detector channel in the **Analysis settings** window.

**Table 28** StepOnePlus threshold values for the Fungiplex Aspergillus IVD PCR Kit

Channel	FAM	JOE	ROX
<b>Target(s)</b>	Ext / PCR Control	<i>Aspergillus</i> spp.	<i>A. terreus</i>
<b>Threshold</b>	500	1000	500

It is important to visually inspect the amplification plots for each sample to ensure that the results recorded are due to true amplification and cannot be attributed to background noise recorded above the defined thresholds.

## 8.7 FluoroCycler® XT

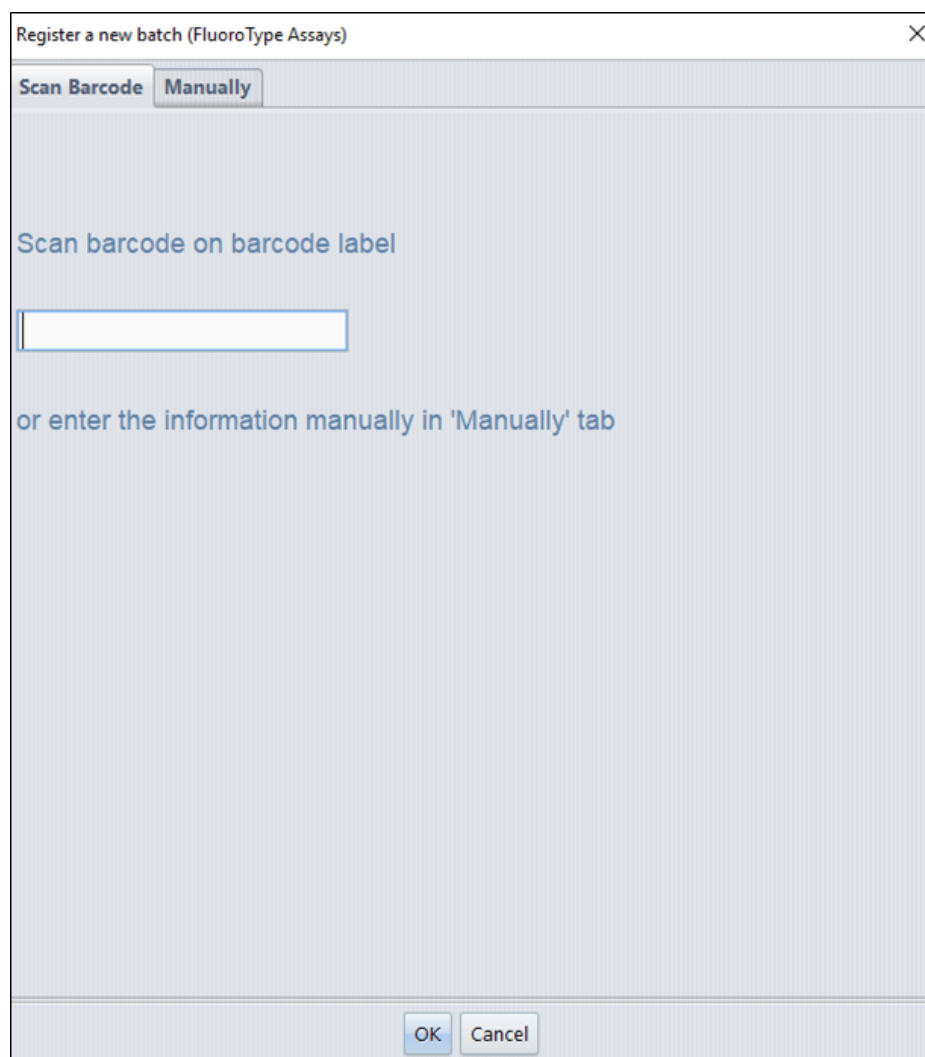
The FluoroCycler® XT has a bespoke software module for running the Fungiplex Aspergillus IVD PCR Kit. The thermal profile, channel selection, data analysis and result interpretation are performed automatically. A report is generated where a result for all wells is displayed.

The manufacturer's instruction manual offers a comprehensive overview of the instrument but a brief summary for running the Fungiplex Aspergillus IVD PCR Kit is detailed below.

Prior to running on this system, the PCR plate must be sealed with 4titude® Clear Weld Seal Mark II seals using a 4titude® Semi-Automatic Sheet Heat Sealer (model - 4s3™).

A barcode is included in the Fungiplex Aspergillus IVD PCR Kit. This is required to register a lot number within the Hain FluoroCycler® XT software. This will be required for every new batch of the kit used on the instrument.

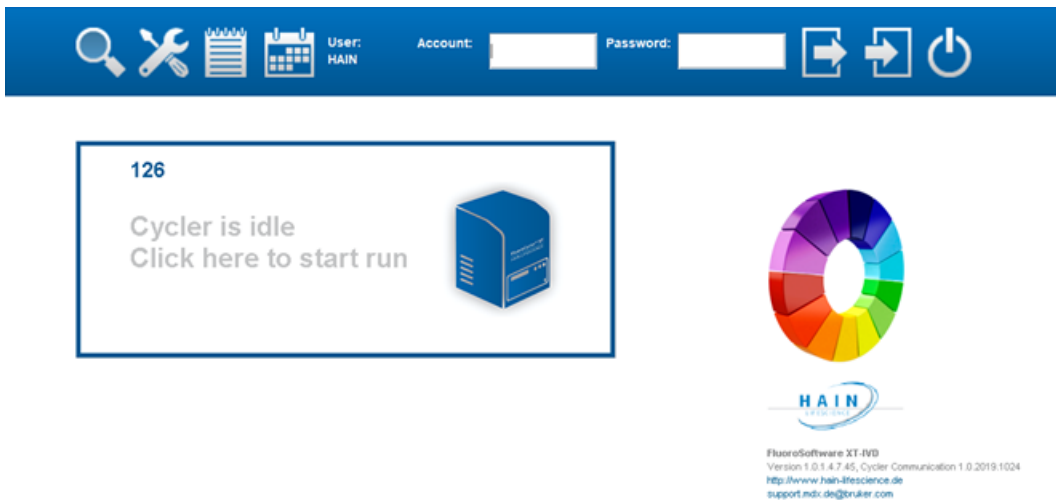
Click the spanner button in the main window and select **Register new batch** from the **FluoroType Assays** tab. A new window opens (see Figure 1).



**Figure 1** Registering a new kit lot number using a barcode scanner

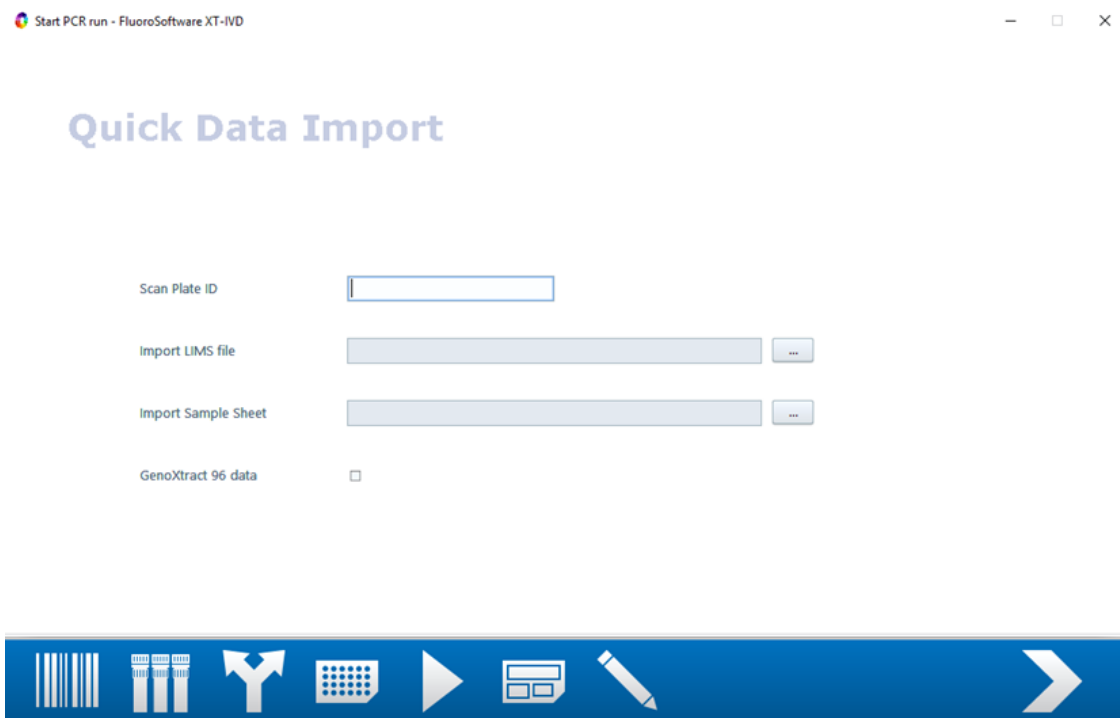
Insert the new kit lot number using the barcode scanner.

When the kit lot has been registered successfully, the message **The Lot No is ready to use** appears. In order to run a plate, select **Click here to start run** on the instrument home page (see Figure 2).



**Figure 2 Instrument home page**

When prompted, enter the plate barcode (found on the side of the PCR plate with the format FCxxxxxx) and click **Next** (see Figure 3).



**Figure 3 Quick Data Import page**

Select **FP Aspergillus** from the **Choose FluoroType Assay** page; then click **Next** (see Figure 4).

## Choose FluoroType Assay

Clear all

FP Aspergillus x

Search for Assay

Bacteria	Fungi	Viruses
Carbaplex 1 Carbaplex 2 MTBDR 2.0 - Clinical Specimen MTBDR 2.0 - Cultivated Sample	FP Aspergillus FP Aspergillus Azole-R FP Candida	BKV SARS-CoV-2 plus



**Figure 4** Choose FluoroType Assay page

The run can be started immediately by selecting the **Start Run first** option; otherwise, select **Enter Sample Data first** (see Figure 5).

**Note** The sample information must be entered before the run is finished. Otherwise, the data will be deleted and no results will be displayed.

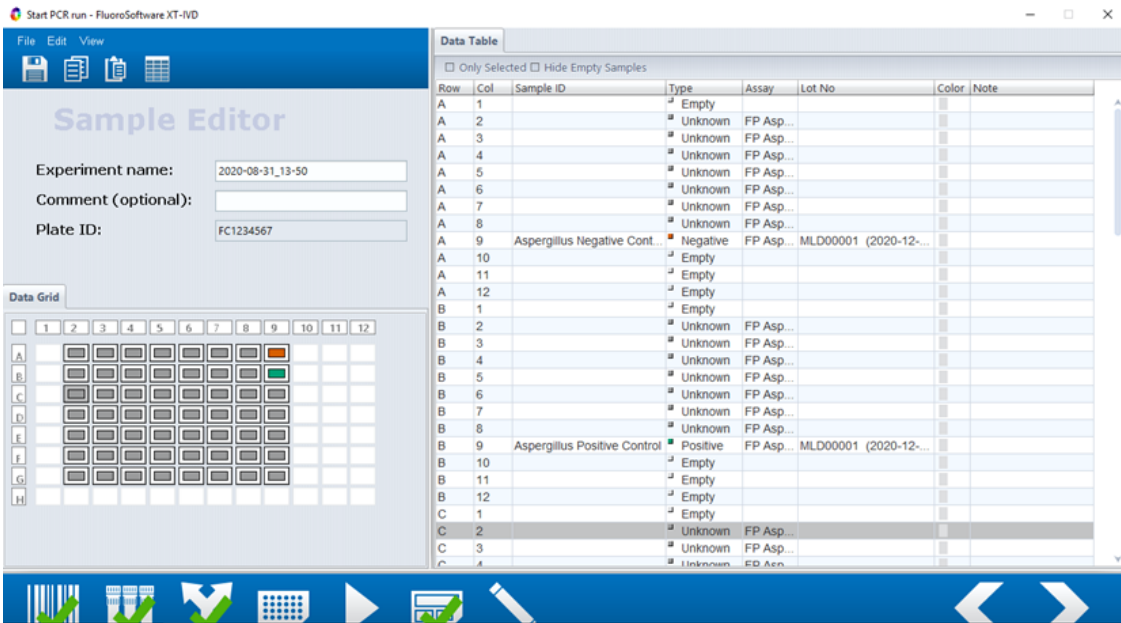
## Choose Run Option

- Start Run first
- Enter Sample Data first



**Figure 5** Choose Run Option page

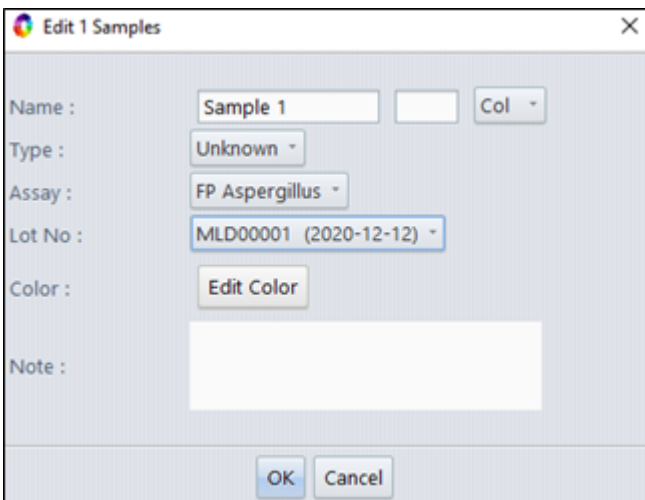
Follow the wizard to open the **Sample Editor** page (see Figure 6).



**Figure 6** Sample Editor page (no special characters can be used here)

Label all samples as either **Unknown**, **Positive** or **Negative** by selecting on the appropriate wells; right click to edit the samples. Ensure **Name**, **Assay Type** and **Lot No** fields are populated as per Figure 7.

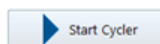
**Note** Only one (1) replicate of positive control and negative control per plate is permitted. The positive control must be the PCR positive control prepared during sample set-up (see section 7). If using the Ext / PCR Control as an extraction control, the negative sample assigned here must be an extracted negative.



**Figure 7** Edit sample window (sample names must be greater than 2 character long)

Complete the wizard set-up, load the plate and start the run (see Figure 8).

## Start Cyclcr



**Figure 8** Start Cyclcr page

Once completed, a PDF report is automatically generated detailing the results for all wells.

It is important to visually inspect the amplification plots, located on the report, for each sample to ensure that the results recorded are due to true amplification and cannot be attributed to background noise recorded above the defined thresholds.



## 8.8 Mic qPCR Cycler

Refer to the manufacturer's instruction manual for information on how to operate the real-time PCR instrument and perform data analysis.

Select the appropriate fluorophore within each channel and assign to the relevant target, as shown in Table 29.

**Table 29** Detector channels used on the Mic qPCR Cycler instrument

	GREEN	YELLOW	ORANGE
<b>Reporter Dye Channel (5' Modifier)</b>	FAM	VIC	ROX
<b>Target detected</b>	Ext / PCR Control	<i>Aspergillus</i> spp.	<i>A. terreus</i>

Configure the Mic qPCR Cycler with the following settings:

- **Temperature Control: Standard TAQ (v3)**
- **Volume: 20 µL**
- **Set to acquire on: Green, Yellow and Orange channels**
- **Auto-gain** for each channel set to **10**, collecting on all tubes

See Table 30 for the thermal profile settings, data should be collected for all 45 cycles.

**Table 30** PCR parameters for the Fungiplex Aspergillus IVD PCR Kit

Step	Temperature (°C)	Time	Number of cycles	Data collection
<b>Activation</b>	95	15 minutes	1	Off
<b>Denaturation</b>	95	5 seconds	45	Off
<b>Annealing / extension</b>	60	30 seconds		On

To analyze the data, apply the following settings to all channels:

- **Exclusion: Extensive**
- **Method: Dynamic**
- **Ignore cycles before: 5**
- **Threshold Start: 1.00**
- Apply the **Threshold Level** and **Fluorescence Cutoff Level** to each channel as shown in Table 31.

**Table 31** Mic qPCR Cycler threshold values for the Fungiplex Aspergillus IVD PCR Kit

	GREEN	YELLOW	ORANGE
<b>Target(s)</b>	Ext / PCR Control	<i>Aspergillus</i> spp.	<i>A. terreus</i>
<b>Threshold Level</b>	0.300	0.700	0.500
<b>Fluorescence Cutoff Level</b>	1%	2%	1%

It is important to visually inspect the amplification plots for each sample to ensure that the results recorded are due to true amplification and cannot be attributed to background noise recorded above the defined thresholds.

## 9 Interpretation of results

### 9.1 Ext / PCR Control

Detection of the Ext / PCR Control is not required with a positive result. In instances where the Ext / PCR Control is negative but the sample has been reported as positive for one or more *Aspergillus* species, the positive result should be considered valid.

#### When using the Ext / PCR Control as a PCR inhibition control

In instances where the sample is reported as negative for all targets and the Ext / PCR Control is also negative, the user should repeat the assay using the same extracts. If the Ext / PCR Control is then reported as positive, it is likely that the original result was due to a handling error / PCR inhibition and any new results should be reported. If the Ext / PCR Control is still reported as negative, the sample should be re-tested, starting from the extraction step.

#### When using the Ext / PCR Control as an extraction control

If the Ext / PCR Control is reported as negative, the sample should be re-tested, starting from the extraction step.

### 9.2 Positive and negative controls

At least one positive and negative control should be included in each analysis. Each negative or positive control should be prepared and tested in the same manner as patient samples.

A negative control that yields a positive test result is indicative of a sample handling or contamination problem. The assay run should be repeated using a fresh aliquot of negative control material, ensuring that the work area and equipment are properly decontaminated and extreme care is taken during PCR set-up.

A positive control that yields a negative result is indicative of reagent failure or sample handling error. Ensure all reagents have been stored correctly and, where applicable, are within expiry date before repeating the assay run taking extreme care during PCR set-up. Expected results for the positive control are provided in Table 32.

**Table 32** Expected positive control results

<b>Positive Control <i>Aspergillus</i></b>	<b>Expected positive targets</b>
	<b><i>Aspergillus</i> spp. POSITIVE + <i>A. terreus</i> POSITIVE</b>

### 9.3 Unknown / patient samples

Always visually inspect the amplification plots for each sample to ensure that the results recorded are due to true amplification and cannot be attributed to background noise recorded above the defined thresholds.

Samples sent for testing via the Fungiplex *Aspergillus* IVD PCR Kit may have significantly varied loads of material present and much of this may be very close to the limit of detection of the biomarker, therefore any  $C_t$  value less than 45 for the corresponding *Aspergillus* species may indicate a positive sample. Table 33 summarizes the results possible with the Fungiplex *Aspergillus* IVD PCR Kit.

**Table 33 Results achieved with Fungiplex Aspergillus IVD PCR Kit**

Channel	Reported target	C <sub>t</sub> value	Result interpretation
Channel 1	Ext / PCR Control	>10	Ext / PCR Control PASS
		N/A or <10	Ext / PCR Control FAIL
Channel 2	<i>Aspergillus</i> spp.	<45	Positive sample
		N/A	Negative sample
Channel 3	<i>A. terreus</i>	<45	Positive sample
		N/A	Negative sample

**IMPORTANT** *A. terreus* positive results may also produce a positive result for *Aspergillus* spp. This is due to the sequence similarity. On occasions where *A. terreus* and *Aspergillus* spp. are both positive, this is most likely due to an *A. terreus* positive sample and does not necessarily infer dual infection.

## 9.4 FluoroCycler® XT results interpretation

Sections 9.1 to 9.3 are also applicable to the FluoroCycler® XT with the F-XT the Ext / PCR Control is called 'Internal Control', however the automatic result processing and PDF report generation mean further definitions are required, as detailed below.

The top of the report will state the negative and positive control result, for example, **1 valid** or **1 invalid**. Table 34 summarizes the numerous possible results with accompanying definitions.

**Table 34 Definitions for FluoroCycler® XT reports**

Result	Explanation
Aspergillus spp.: positive (CP=xx.x)	<i>Aspergillus</i> spp. DNA was detected in the sample at stated CP.
Aspergillus terreus: negative	
Aspergillus spp.: negative	<i>A. terreus</i> DNA was detected in the sample at stated CP. <b>IMPORTANT</b> <i>A. terreus</i> positive results may also produce a positive result for <i>Aspergillus</i> spp. This is due to the sequence similarity. On occasions where <i>A. terreus</i> and <i>Aspergillus</i> spp. are both positive, this is most likely due to an <i>A. terreus</i> positive sample and does not necessarily infer dual infection.
Aspergillus terreus: positive (CP=xx.x)	
Aspergillus spp.: negative	No target DNA detected in the sample.
Aspergillus terreus: negative	
Aspergillus spp.: Invalid	The CP value of <i>Aspergillus</i> spp. is not within the validated range.
Aspergillus terreus: Invalid	The CP value of <i>A. terreus</i> is not within the validated range.
Invalid: Internal Control Inhibited	Sample was negative for the presence of target DNA and the Ext / PCR Control was detected with a CP value which indicates inhibition.

<b>Result</b>	<b>Explanation</b>
Invalid: Internal Control out of range	Sample was negative for the presence of target DNA and the Ext / PCR Control was not detected within the validated CP range.
FP Aspergillus Negative Control: 1 valid	Only the Ext / PCR Control was detected in the negative control.
FP Aspergillus Positive Control: 1 valid	<i>Aspergillus</i> spp. and <i>A. terreus</i> were detected in the positive control.
FP Aspergillus Negative Control: 1 invalid	No Ext / PCR Control was detected in the negative control and / or contamination was detected in the negative control.
FP Aspergillus Positive Control: 1 invalid	No <i>Aspergillus</i> spp. and / or <i>A. terreus</i> was detected in the positive control.

**Note** For the FluoroCycler® XT stricter interpretation ranges are used in order to provide a more reliable result

## 10 Performance characteristics

The performance characteristics identified from validation of the Fungiplex Aspergillus IVD PCR Kit are highlighted in Table 35 and Table 36. Table 35 provides the analytical performance with known quantities of material analyzed at the limit of detection (LOD) of 20 input copies and above (DNA), while Table 36 presents the clinical performance data from a multi-centre performance evaluation.

**Table 35 Analytical performance characteristics of the Fungiplex Aspergillus IVD PCR Kit**

	<b>Sensitivity (%)</b>	<b>Specificity (%)</b>
<b>Contrived samples</b>	<b>99.33</b>	<b>98.53</b>

**Table 36 Clinical performance characteristics of the Fungiplex Aspergillus IVD PCR Kit**

<b>Sample type</b>		<b>Single result</b>	<b>Duplicate result</b>
<b>Simulated serum samples</b>	Sensitivity (%)	<b>95.83</b>	-
	Specificity (%)	<b>96.49</b>	-
<b>Simulated BAL samples</b>	Sensitivity (%)	<b>85.00</b>	-
	Specificity (%)	<b>96.43</b>	-
<b>Retrospective clinical samples</b>	Sensitivity (%)	<b>95.24</b>	<b>70.00</b>
	Specificity (%)	<b>89.66</b>	<b>100.00</b>
<b>Prospective clinical samples</b>	Sensitivity (%)	<b>N/A</b>	<b>N/A</b>
	Specificity (%)	<b>90.18</b>	<b>98.55</b>

Figures for clinical performance are based on:

- Simulated serum samples analyzed over a concentration range of 1 – 1000 CFU/ 0.5 mL
- Simulated BAL samples analyzed over a concentration range of 1 – 100 genomes and 1 – 100 conidia/0.5 mL
- Retrospective testing was conducted on 274 samples obtained from 41 patients categorized as Proven/Probable IA, or 'no evidence of fungal disease' (according to EORTC criteria)
- Prospective testing was conducted on 132 samples obtained from 65 hematology patients considered 'at-risk' of IA

# 11 Disposal

## General disposal



Dispose of any unused reagents, waste and shipping materials in accordance with country, state and local regulations.

## Dry ice disposal



Dry ice contains CO<sub>2</sub> and must be handled with gloves in a well ventilated area. Dispose of any dry ice in accordance with country, state and local regulations.

## Packaging disposal














Please dispose of all packaging in accordance with local recycling regulations.

The above instructions for disposal apply to EU member states. Correct disposal of this product will help conserve natural resources and ensure that it is recycled in a manner that prevents potential negative consequences for the environment and human health. For more information, please contact your local waste disposal service.

## 12 Symbols

The following symbols are used in the labeling:

	Catalogue number
	CE marking
	<i>In vitro</i> diagnostic medical device
	Manufacturer
	Temperature limit
	Batch code
	Use-by date
	Contains sufficient for <n> tests
	Software version number
	Unique product identifier
	Consult instructions for use

## 13 Manufacturer



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