sartorius

Instructions for Use

Microsart® ATMP Fungi

Fungi and yeast Detection Kit for qPCR

Prod. No. SMB95-1012

Reagents for 100 reactions

For use in research and quality control

Manufactured by:

Minerva Biolabs GmbH
Schkopauer Ring 13
12681 Berlin
Germany

Symbols

Symbol	Meaning
LOT	Lot No.
REF	Order No.
	Expiry date
	Store at
	Contains reagents for 100 tests
	Manufacturer

Contents

1. Intended Use **5**

2. Explanation of the Test **5**

3. Test Principle **5**

4. Notes on the Test Procedure **6**
4.1 Handling and Equipment Recommendations . . 6

5. Reagents **7**

6. Needed but not Included **8**
Consumables 8
Equipment 8

7. Specimen **10**

8. Test Procedure **10**
8.1 Recommendation for product release testing 10
8.2 Rehydration of the Reagents 11
8.3 Preparation of the Reaction Mix 12
8.4 Loading the Test Tubes 12
8.5 Starting the Reaction 13
8.6 Analysis 13

9. Short Instructions **14**

10. Interpretation of Results **15**
10.1 Yes/No Evaluation 15
10.2 Total Analysis and recommended actions for product release testing 16

11. Appendix **17**
QuantStudio™ 17
Bio-Rad CFX96 Touch™ / CFX96 Touch™ deep well . 19
Rotor-Gene® 6000 (5-plex) 22
ABI Prism® 7500 24
Mx3005P™ 26

12. Related products **28**

1. Intended Use

Microsart® ATMP Fungi kit is designed for the direct detection of fungi and yeasts in cell cultures, cell culture derived biologicals and Advanced Therapy Medicinal Products (ATMPs, e.g. autologous transplants), based on real-time PCR (qPCR).

2. Explanation of the Test

Microsart® ATMP Fungi utilizes qPCR as the method of choice for sensitive and robust detection of fungal contamination. The assay can be performed with any type of real-time PCR cycler able to detect the fluorescent dyes FAM™ and ROX™.
The detection procedure can be performed within 2.5 hours (including DNA extraction: 3.5 hours). In contrast to the culture method, samples do not need to contain living particles.

3. Test Principle

Fungi and yeasts are specifically detected by amplifying a highly conserved rRNA operon, or more specifically, a 18S rRNA coding region in their genome. The specific amplification is detected at 520 nm (FAM™ channel). The kit includes primers and FAM™ labeled probes, which allow the specific detection of many fungal species.
The Fungi Mix contains the polymerase. Prokaryotic DNA is not amplified by this primer/ probe system. False negative results due to PCR inhibitors or improper DNA extraction are detected by using the internal amplification control. The Internal Control DNA can be added directly to the PCR master mix to act as a PCR control or used to monitor the extraction process. The amplification of the internal amplification control is detected at 610 nm (ROX™ channel).

4. Notes on the Test Procedure

1. For in vitro use in research and quality control. This kit may be disposed of according to local regulations.
2. This kit should be used only by trained persons. You should wear a clean lab coat and use disposable gloves at all times while performing the assay.
3. To avoid DNA cross-contaminations, the complete test must be performed under sterile and DNA-free conditions (see chapter 4.1 for detailed information).
4. In case of working with living fungal strains, the local regulatory requirements for S2 labs must be considered.
5. Attention: by aliquoting and freezing your samples you run a high risk of contamination. This should therefore be avoided if possible.
6. This leaflet must be widely understood for a successful use of Microsart® ATMP Fungi. The reagents supplied should not be mixed with reagents from different lots but used as an integral unit. The reagents of the kit should not be used beyond their shelf life.
7. Any deviation from the test method can affect the results.
8. Inhibition may be caused by the sample matrix, but also by sample elution buffer of DNA extraction kits which are not recommended or validated. Please note that by using DNA extraction kits which are not validated you run a high risk of obtaining false-positive or false-negative results.
9. For each test setup, at least one negative control should be included. Positive controls facilitate the evaluation of the test. Typical Ct values for the internal control and positive control are shown on the Certificate of Analysis and can be used as a guideline for quality control.
10. The use of control samples is advised to secure the day-to-day validity of results. The controls should be carried out in the same manner as the samples.
11. Participation in external quality control programs, such as those offered by Minerva Biolabs GmbH (www.minerva-biolabs.com), is recommended.

4.1 Handling and Equipment Recommendations

To avoid false positive results due to improper handling the following actions are recommended:

1. To avoid DNA cross contaminations the complete test must be performed under sterile and DNA free conditions. Therefore, we recommend the use of an isolator/

glovebox with an airlock.
2. The isolator/glovebox should be cleaned thoroughly with PCR Clean™ (Minerva Biolabs, Prod. No. 15-2025) or PCR Clean™ Wipes (Minerva Biolabs, Prod. No. 15-2001) before and during the working process.
3. All materials, which are introduced into the isolator/glovebox should be cleaned thoroughly with PCR Clean™. Don´t forget to clean the airlock with PCR Clean™. Pipettes and gloves should be cleaned thoroughly with PCR Clean™ Wipes prior and during the process.
4. Avoid working above open tubes and avoid air turbulences due to rapid movements.
5. Be careful when opening the tubes. Do not touch the inner surface of the lid.

5. Reagents

Each kit contains reagents for 100 reactions. The expiry date of the unopened package is marked on the package label. The kit components are stored at +2 to +8°C until use. Lyophilized components must be stored ≤ -18 ° C after rehydration. Protect the Fungi Mix from light.

Kit Component Label Information	100 Reactions Order No. SMB95-1012	Cap Color
Fungi Mix	4 x lyophilized	orange
Rehydration Buffer	4 x 0.5 ml	blue
Positive Control DNA	1 x lyophilized	green
Internal Control DNA	4 x lyophilized	yellow
PCR grade Water	5 x 1.5 ml	white

The lot specific Certificate of Analysis can be downloaded from the manufacturer's website (www.minerva-biolabs.com).

6. Needed but not Included

Microsart® ATMP Fungi contains the reagents, including negative and positive controls and polymerase as a component of the Fungi Mix, to perform the test.
General industrial supplies and reagents, usually available in PCR laboratories are not included:

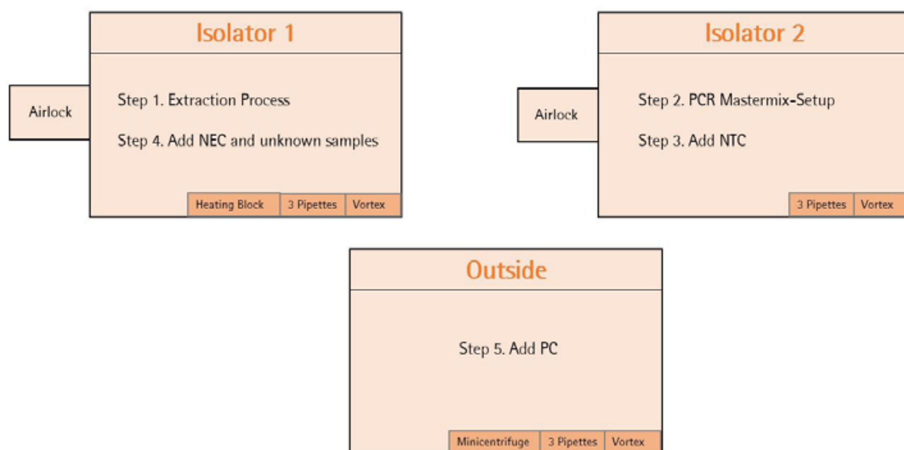
Consumables
- Laboratory gloves
- PCR Clean™ (Minerva Biolabs, Prod. No. 15-2025) and PCR Clean™ wipes (Minerva Biolabs, Prod. No. 15-2001)
- DNA-free PCR reaction tubes (PCR 8-SoftStrips with attached caps from Biozym are recommended: 0.1 ml Low Profile, Prod. No. 710975 and 0.2 ml High Profile, Prod. No. 710970)
- DNA-free pipette filter tips that must be free from Fungi DNA (Biosphere® filter tips from Sarstedt are recommended: 0.5-20 µl, Prod. No. 70.1116.210; 2-100 µl, Prod. No. 70.760.212; 20-300 µl, Prod. No. 70.765.210; 100-1000 µl. Prod. No. 70.762.211)
- Microsart® ATMP Extraction kit, a DNA-free extraction kit, Sartorius Prod. No. SMB95-2001.

Equipment
- Isolator/glovebox (further information, supplier and prices are available on request, please contact PCR@sartorius.com)
- qPCR device with filter sets for the detection of the fluorescent dyes FAM™ and ROX™ and suitable for 25 µl PCR reaction volumes
- Minicentrifuge for reaction tubes and PCR tubes
- Vortex mixer
- Rack for 1.5 ml tubes and for PCR-tube strips
- Pipettes (Sartorius)
  Mechanical
  - 0.5 – 10 µl Sartorius Prod. No. LH-729020
  - 10 – 100 µl Sartorius Prod. No. LH-729050
  - 100 – 1000 µl Sartorius Prod. No. LH-729070

  or electrical
  - 0.2 – 10 µl Sartorius Prod. No. 735021
  - 10 – 300 µl Sartorius Prod. No. 735061
  - 50 – 1000 µl Sartorius Prod. No. 735081

If you use the Microsart® ATMP Extraction kit you additionally need the following equipment:
- Isolator/glovebox (for extraction process)
- Vortex mixer
- Set of 3 pipettes (10 µl, 100 µl and 1000 µl; see page 8 for order information)
- Microcentrifuge for 1.5 ml reaction tubes (Centrisart A-14, Sartorius Prod. No. A-14-1EU)
- Heat block with optional shaking function, when performing DNA extraction prior to PCR
- Rack for 1.5 ml tubes

Schematical overview of technical setup and experimental design:



It is also possible to connect Isolator 1 and Isolator 2 via an airlock so that you can transfer the PCR-tubes after Step 3 directly from Isolator 2 into Isolator 1.
Please note that in this case you would need an additional airlock for Isolator 2.

7. Specimen

Studies showed the strict requirement of DNA extraction for any kind of sample to achieve highest sensitivity. For most test materials, a DNA extraction method is available providing templates suitable for PCR. However, most of the DNA extraction kits available on the market are not free of DNA contaminations. Therefore, we recommend the combination of Microsart® ATMP Fungi with the Microsart® ATMP Extraction kit (Prod. No. SMB95-2001), a unique state-of-the-art DNA extraction method, which eliminates the risk of DNA contaminations, facilitating the detection of fungi and yeasts in cell culture and ATMPs via qPCR.

8. Test Procedure

The test should be carried out with negative and positive controls and samples in duplicates. For quantification, a dilution series of an appropriate standard is prepared. To this aim, Microsart® Calibration Reagents are recommended (see chapter 12: Related Products for ordering information). All reagents and samples must be equilibrated at +2 to +8 °C prior to use.

8.1 Recommendation for product release testing

The extraction process (Microsart® ATMP Extraction) should be carried out with a negative extraction control (NEC) and samples in duplicates. Additionally, the PCR test should include a PCR negative control (no-template control, NTC) and a PCR positive control.

DNA extraction	PCR
2 x Sample	2 x Sample
1 x Negative Extraction Control	1 x Negative Extraction Control
	1 x PCR Positive Control
	1 x PCR Negative Control
∑ 3 extractions	∑ 5 PCR reactions

8.2 Rehydration of the Reagents

After reconstitution, reagents must be stored at ≤ -18 °C. In order to avoid repeated freezing and thawing, storage in appropriate aliquots is recommended.

1.	Fungi Mix Internal Control DNA Positive Control DNA	orange cap yellow cap green cap	Centrifuge briefly
2.	Fungi Mix	orange cap	Add 390 µl Rehydration Buffer (blue cap)
3.	Internal Control DNA	yellow cap	Add 800 µl PCR grade Water (white cap)
4.	Positive Control DNA	green cap	Add 300 µl PCR grade Water (white cap)
5.	Fungi Mix Internal Control DNA Positive Control DNA	orange cap yellow cap green cap	Incubate 5 min at room temperature
6.	Fungi Mix Internal Control DNA Positive Control DNA	orange cap yellow cap green cap	Vortex briefly

8.3 Preparation of the Reaction Mix

Preparation of the master mix and sample loading should not take more than 45 minutes to avoid a reduction in the fluorescent signal. The pipetting sequence should be respected and the tubes closed after each sample has been loaded.
The total volume per reaction is 25 µl including 10 µl sample. Calculations of reaction setup should include positive (PC) and negative controls (NTC). If the Internal Control DNA was not added to the sample to monitor the DNA extraction process, follow this protocol:

1. Prepare the master mix at room temperature, by adding 26 µl of Internal Control DNA (yellow cap) directly in the Fungi Mix tube (orange cap).
2. Homogenize the reaction mix by tapping carefully against the tube. Spin briefly.
3. Add 15 µl of the master mix to 25 PCR tubes. Close PCR tubes. For storage, freeze any master mix aliquots you do not need for the current assay.
   Attention: in order to avoid contaminations we recommend to freeze the PCR tubes containing the master mix in a closed and clean PCR rack.

Attention:
If the Internal Control DNA was added to the sample during DNA extraction, add 15 µl of the Fungi Mix (orange cap) directly to each PCR tube.

Attention:
Don´t forget to add 1 µl of Internal Control DNA to NTC and PC.

8.4 Loading the Test Tubes

1. Negative controls: add 10 µl Suspension Buffer (violet cap) from the DNA extraction kit or PCR grade Water (white cap). Seal tube before proceeding with the samples.
   Attention: in order to avoid contaminations make sure to reserve one of the PCR grade water tube (white cap) exclusively to the negative controls.
2. Sample reaction: add 10 µl of sample. Seal tube tightly before proceeding.
   Attention: Samples, including NECs, should be added to the reaction in the isolator/glovebox used for DNA extraction.

3. Positive control: add 10 µl Positive Control DNA (green cap).
   Attention: Positive controls should not be handled in the isolator/glovebox used for master mix setup or DNA extraction.
4. Close and spin all PCR tubes briefly, load the qPCR cycler and start the program.

8.5 Starting the Reaction

1. Load the cycler, check each PCR tube and the cycler lid for tight fit.
2. Program the qPCR cycler or check stored temperature profiles. See Appendix for temperature profiles of selected qPCR cyclers.
3. Start the program and data reading.

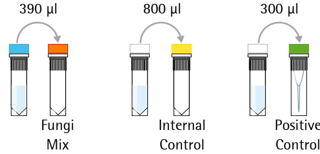
8.6 Analysis

1. Save the data at the end of the run.
2. Analyze the channels for the fluorescent dyes FAM™ and ROX™.
3. FAM™: adapt the threshold line to 10 % of the maximum fluorescence level of the positive controls.
   ROX™: adapt the threshold line to 10 % of the maximum fluorescence level of the NTCs. In case of duplicate determination take the average of the maximum fluorescence levels. See chapter 11.
4. Analyze the calculation of the Ct-values for negative controls, positive controls and samples.

9. Short Instructions

1. Rehydration of Reagents

centrifuge Fungi Mix
centrifuge Positive Control DNA
centrifuge Internal Control DNA



incubate for 5 min RT
vortex briefly
centrifuge for 5 sec

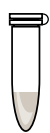
2. Preparation of PCR Reaction

a) Internal Control added during DNA extraction

+ 15 µl Fungi Mix (orange cap)

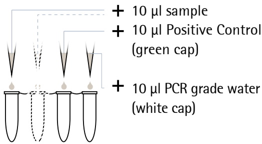
don't forget to add 1 µl Internal Control to NTC and PC

b) Internal Control not added during DNA extraction



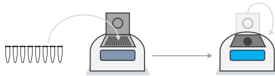
Reaction Mix
+ 15 µl Fungi Mix
+ 1 µl Internal Control

+ 15 µl Reaction Mix

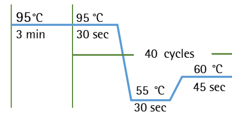


+ 10 µl sample
+ 10 µl Positive Control (green cap)
+ 10 µl PCR grade water (white cap)

3. Starting PCR Reaction



Start PCR program



Colour	Reagent
blue	Rehydration Buffer
orange	Fungi Mix
white	PCR grade water
green	Positive Control
yellow	Internal Control

- incubate
- vortex
- centrifuge
- + add

storage 2-8 °C
after rehydration ≤ -18 °C

This procedure overview is not a substitute for the detailed manual.

10. Interpretation of Results

The presence of fungal DNA in the sample is indicated by an increasing fluorescence signal in the FAM™ channel during PCR. The concentration of the contaminant can be calculated by a software comparing the crossing cycle number of the sample with a standard curve created in the same run.

A successfully performed PCR without inhibition is indicated by an increasing fluorescence signal in correspondence of the internal control channel. Fungal DNA and Internal Control DNA are competitors in PCR. Because of the very low concentration of Internal Control DNA in the PCR mix, the signal strength in this channel is reduced with increasing fungal DNA loads in the sample.

10.1 Yes/No Evaluation

Detection of Fungi FAM™ channel	Internal Control ROX™ channel	Interpretation
positive ($C_t < 40$)	irrelevant	Fungi/yeast positive
negative (no C_t)	negative**	PCR inhibition*
negative (no C_t)	positive ($C_t < 40$)	Fungi/yeast negative

*PCR inhibition might be caused by sample matrix. If one out of two replicates is negative for Internal Control (ROX™: no Ct), repeat the PCR. If two out of two replicates are negative for the Internal Control, extract DNA from your sample material and repeat the PCR.

** if used as PCR control, Internal control of fungi negative samples (FAM™: no Ct) must show Ct-values in the range of +/- 2 cycles (ROX™) of the negative control (master mix control, NTC). If used as process control, Internal Control of fungi negative samples (FAM™: no Ct) must show Ct-values in the range of +/- 3 cycles (ROX™) of the NTC.

10.2 Total Analysis and recommended actions for product release testing

Sample	Result	Interpretation	Action
NTC	NTC negative	Valid PCR	Interpret specimen results
	NTC positive	PCR contamination	Repeat PCR only
PC	PC positive	Valid PCR	Interpret specimen results
	PC negative	Failed PCR	Repeat PCR only
NEC	NEC negative	Valid PCR	Interpret specimen results
	NEC positive	Contamination during extraction or PCR	Repeat the whole process incl. DNA extraction and PCR
Specimen	0/2 positive	No contamination	Release
	1/2 positive	Possible contamination	Repeat the whole process* incl. DNA extraction, PCR, and analysis. If result is confirmed, no release.
	2/2 positive	Contamination	No release

* If NTC is also positive, repeat PCR only.
In case you want to identify a positive result, please send your PCR product to Minerva Biolabs GmbH. The PCR product will be purified by Minerva Biolabs. Sequencing will be done by an external sequencing service. The interpretation of your sequencing results will be supplied by Minerva Biolabs afterwards.

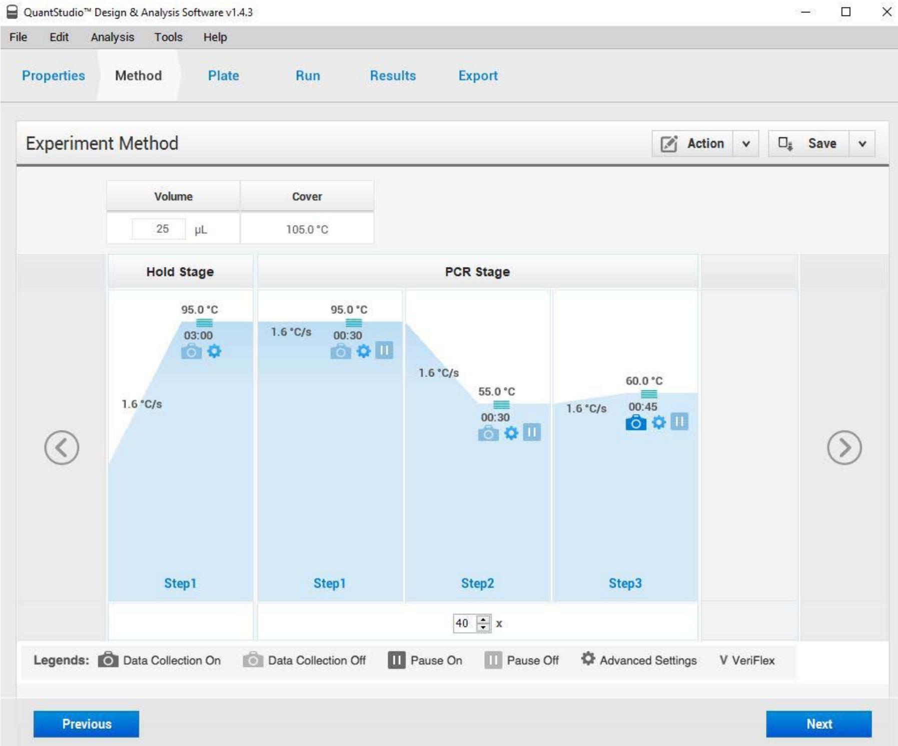
Attention:
In case of a light or multiple contamination, the sequencing analysis might lead to wrong identification.

11. Appendix

The protocol can be performed with any type of real-time PCR cycler able to detect the fluorescent dyes FAM™ and ROX™. The following qPCR cyclers were used for the validation of Microsart® ATMP Fungi:
QuantStudio™, Mx3005P™, CFX96 Touch™, CFX96 Touch Deep Well™, ABI Prism® 7500, Rotor-Gene® Q/Rotor-Gene®.
In addition, Microsart® ATMP Fungi kit was successfully tested with the LightCycler® 480 II (protocol available on request).

QuantStudio™

- Click File --> New Experiment --> Experiment Setup, to open the setup menu.
- Select “Properties” from the menu bar to open the “Experiment Properties” tab. In this tab, assign an experiment name (“Name”) and make sure that “Chemistry” is set to “TaqMan® Reagents” and that the “Run mode” option is set to “Standard”
- Select “Method” in the menu bar to open the “Experiment Method” tab.
  Adjust the reaction volume and the cover temperature by setting “Volume” to 25 µl and “Cover” to 105 °C.
  Program a “Hold Stage” of 3 min at 95 °C.
  In “PCR Stage”, perform “Step1” (denaturation step) at 95 °C for 30 sec, “Step2” (annealing step) at 55 °C for 30 sec, and “Step3” (elongation step) at 60 °C for 45 sec. Enable the data readout during the elongation step by clicking on the camera symbol. Set the number of cycles to 40.
  See also figure below for an overview of these settings.
- Select “Plate” in the menu bar to open the “Assign Targets and Samples” tab.
  Important: Set the “Passive Reference” to “None”!!!!
- Click on the “Advanced Setup” tab to define your targets and samples.
- In the “Targets” section: for the assay target, choose FAM as a reporter and NFQ-MGB as a quencher. For the internal control target, choose ROX as a reporter and NFQ-MGB as a quencher.
- Enter your samples names in the “Samples” section.
- Select the wells containing your samples (from the graphical overview of the plate), enable the targets, and assign the appropriate sample name by clicking on the respective check boxes.
- Select “Run” from the menu bar and click on START RUN to start the PCR run.



Data Analysis

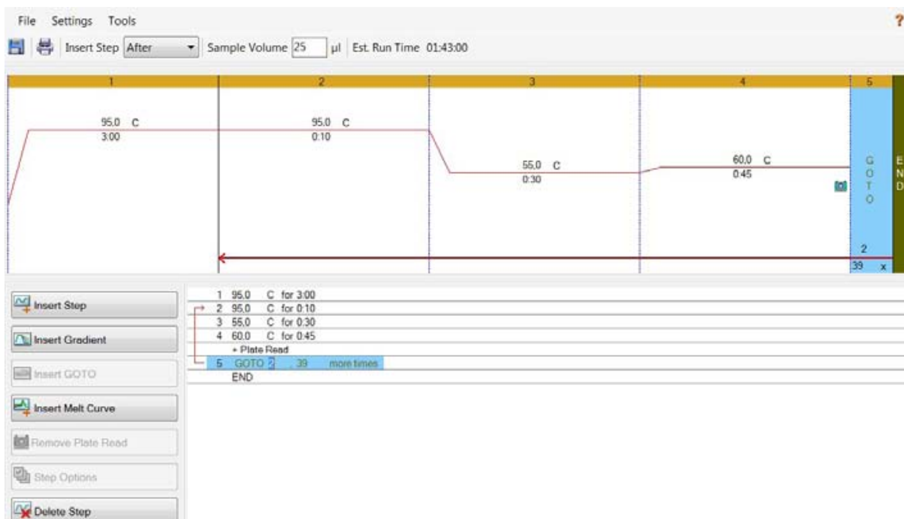
- To enable thresholds setting, please follow the specific instructions provided in the manual of your cycler.
- FAM™: adapt the threshold line to 10 % of the maximum fluorescence level of the positive control.
  ROX™: adapt the threshold line to 10 % of the maximum fluorescence level of the NTCs. In case of duplicate determination take the average of the maximum fluorescence levels.
- Select the Results tab to view specific Ct values

Bio-Rad CFX96 Touch™ / CFX96 Touch™ deep well

Run Setup Protocol Tab:

- Click File --> New --> Protocol to open the Protocol Editor and create a new protocol
- Select any step in either the graphical or text display
- Click the temperature or well time to directly edit the value

Segment 1:	1 cycle	3 min	95 °C	
Segment 2:		30 sec	95 °C	
Segment 3:		30 sec	55 °C	
Segment 4:		45 sec	60 °C	data collection
		GOTO Step 2, 39 more cycles		



Run Setup Plate Tab:

- Click File --> New --> Plate to open the Plate Editor and create a new plate
- Specify the type of sample at Sample Type
- Name your samples at Sample Name
- Use the Scan Mode dropdown menu in the Plate Editor Toolbar to designate the data acquisition mode to be used during the run. Select All Channels mode
- Click Select Fluorophores to indicate the fluorophores that will be used in the run. Choose FAM™ for the detection of fungi amplification and ROX™ for monitoring the amplification of the internal control. Within the plate diagram, select the wells to load
- Choose the fluorophore data you want to display by clicking the fluorophore checkboxes located under amplification chart. Select FAM™ to display data of fungi detection and ROX™ to display internal control amplification data.

Data Analysis:

- Select Settings in the menu and select Baseline Subtracted Curve Fit as baseline setting and Single Threshold mode as Cq determination
- Remark: Amplification curves for which the baseline is not correctly calculated by the software, can be manually adapted
- To enable thresholds setting, please follow the specific instructions provided in the manual of your cycler.
- FAM™: adapt the threshold line to 10 % of the maximum fluorescence level of the positive controls.
  ROX™: adapt the threshold line to 10 % of the maximum fluorescence level of the NTCs. In case of duplicate determination take the average of the maximum fluorescence levels.
- Evaluate the Ct-values according to chapter 10

Rotor-Gene® 6000 (5-plex)

For the use of Rotor-Gene® 6000, 0.1ml PCR tubes from Qiagen are recommended (Prod. No. 981106). Those tubes shall imperatively be used with the 72 well rotor from Rotor-Gene® 6000.

1. Check the correct settings for the filter combination:

Target	Fungi	Internal Control
Filter	green	orange
Wavelength	470—510 nm	585-610 nm

2. Program the Cycler:

Program 1: Pre-incubation

Setting	Hold
Hold Temperature	95 °C
Hold Time	3 min 0 sec

Program Step 2: Amplification

Setting	Cycling
Cycles	40
Denaturation	95 °C for 30 sec
Annealing	55 °C for 30 sec
Elongation	60 °C for 45 sec —> acquiring to Cycling A (green and orange)
Gain setting	automatic (Auto-Gain)
Slope Correct	activated
Ignore First	deactivated

Analysis:

- Open the menu Analysis
- Select Quantitation
- Check the required filter set (green and orange) according to the following table and start data analysis by double click.
- The following windows will appear:
  Quantitation Analysis - Cycling A (green / orange)
  Quant. Results - Cycling A (green / orange)
  Standard Curve - Cycling A (green / orange)
- In window Quantitation Analysis, select first "Linear Scale" and then "Slope Correct".
  Threshold setup (not applicable if a standard curve was carried with the samples and auto threshold was selected):
- In window "CT Calculation" set the threshold value to 0-1
- Pull the threshold line into the graph. For FAM™: adapt the threshold line to 10 % of the maximum fluorescence level of the positive controls.
  For ROX™: adapt the threshold line to 10 % of the maximum fluorescence level of the NTCs. In case of duplicate determination take the average of the maximum fluorescence levels.
- The Ct-values can be taken from the window Quant. Results.
- Samples showing no Ct-value can be considered as negative.

ABI Prism® 7500

1. Check the correct settings for the filter combination:

Target	Fungi	Internal Control
Filter	FAM™	ROX™
Wavelength	470-510 nm	585-610 nm
Quencher	none	none

Important:

The ROX™ Reference needs to be disabled. Activate both detectors for each well. Measurement of fluorescence during extension.

2. Program the Cycler:

Program Step 1: Pre-incubation

Setting	Hold
Temperature	95 °C
Incubation time	3 min

Program Step 2: Amplification

Cycles	40
Setting	Cycle
Denaturing	95 °C for 30 sec
Annealing	55 °C for 30 sec
Extension	60 °C for 45 sec

Analysis:

- Enter the following basic settings at the right task bar:
  Data: Delta RN vs. Cycle
  Detector: FAM™ and ROX™
  Line Colour: Well colour
- Open a new window for the graph settings by clicking the right mouse button
  Select the following settings and confirm with ok:
  Real Time Settings: Linear
  Y-Axis Post Run Settings: Linear and Auto
  Scale X-Axis Post Run Settings: Auto Scale
  Display Options: 2
- Initiate the calculation of the Ct-values and the graph generation by clicking on "Analyse" within the report window.
- Pull the threshold line into the graph. For FAM™: adapt the threshold line to 10 % of the maximum fluorescence level of the positive controls.
  For ROX™: adapt the threshold line to 10 % of the maximum fluorescence level of the NTCs. In case of duplicate determination take the average of the maximum fluorescence levels.
- Samples showing no Ct-value can be considered as negative

Mx3005P™

– Go to the setup menu, click on "Plate Setup", check all positions which apply
– Click on "Collect Fluorescence Data" and check FAM™ and ROX™
– Corresponding to the basic settings the "Reference Dye" function should be deactivated
– Specify the type of sample (no template control or positive control, sample, standard) at "well type"
– Edit the temperature profile at "Thermal Profile Design":

Segment 1: 1 cycle	3 min	95 °C
Segment 2: 40 cycles	30 sec	95 °C
	30 sec	55 °C
	45 sec	60 °C data collection end

– at menu "Run Status" select "Run" and start the cycler by pushing „Start"

Analysis of raw data:
– In the window "Analysis" tab on "Analysis Selection / Setup" to analyse the marked positions
– Ensure that in window "algorithm enhancement" all options are activated:
Amplification-based threshold
Adaptive baseline
Moving average
– Click on "Results" and "Amplification Plots". The Threshold will be generated automatically
- FAM™: adapt the threshold line to 10 % of the maximum fluorescence level of the positive controls.
ROX™: adapt the threshold line to 10 % of the maximum fluorescence level of the NTCs. In case of duplicate determination take the average of the maximum fluorescence levels.
- Read the Ct-values in "Text Report"
- Evaluate the Ct-values according to chapter 10

Appendix

Limited Product Warranty
This warranty limits our liability for replacement of this product. No warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided. Minerva Biolabs shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

Trademarks
LightCycler is a registered trademark of a member of the Roche Group. TaqMan is a registered trademark of of Roche Molecular Systems, Inc. ABI Prism is a registered trademark of Applera Corporation or its subsidiaries in the US and certain other countries. FAM and ROX are trademarks of Applera Corporation or its subsidiaries in the US and certain other countries. Mx3005P is a trademark of Agilent Technologies. CFX96 Touch is a trademark of Bio-Rad Laboratories, Inc. QuantStudio is a trademark of Life Technologies Corporation. Rotor-Gene is a registered trademark of Qiagen GmbH. Microsart is a registered trademark of Sartorius Stedim Biotech. PCR Clean is a trademark of Minerva Biolabs GmbH.

Last technical revision: 2019-11-15

12. Related products

Detection Kits for qPCR

SMB95-1001/1002	Microsart® AMP Mycoplasma	25/100 tests
SMB95-1003/1004	Microsart® ATMP Mycoplasma	25/100 tests
SMB95-1005/1006	Microsart® RESEARCH Mycoplasma	25/100 tests
SMB95-1009	Microsart® RESEARCH Bacteria	25 tests
SMB95-1007	Microsart® ATMP Sterile Release	10 samples
SMB95-1014/1013	Microsart® RESEARCH Fungi	25/100 tests

Microsart® Calibration Reagent, 1 vial, 10^8 genomes / vial (bacteria, including Mollicutes)

SMB95-2021	Mycoplasma arginini
SMB95-2022	Mycoplasma orale
SMB95-2023	Mycoplasma gallisepticum
SMB95-2024	Mycoplasma pneumoniae
SMB95-2025	Mycoplasma synoviae
SMB95-2026	Mycoplasma fermentans
SMB95-2027	Mycoplasma hyorhinis
SMB95-2028	Acholeplasma laidlawii
SMB95-2029	Spiroplasma citri
SMB95-2030	Bacillus subtilis
SMB95-2031	Pseudomonas aeruginosa
SMB95-2032	Kocuria rhizophila
SMB95-2033	Clostridium sporogenes
SMB95-2034	Bacteroides vulgatus
SMB95-2035	Staphylococcus aureus
SMB95-2036	Mycoplasma salivarium

Microsart® Calibration Reagent, 1 vial, 10^6 genomes / vial (fungi)

SMB95-2044	Candida albicans
SMB95-2045	Aspergillus brasiliensis
SMB95-2046	Aspergillus fumigatus
SMB95-2047	Penicillium chrysogenum
SMB95-2048	Candida glabrata
SMB95-2049	Candida krusei
SMB95-2050	Candida tropicalis

Microsart® Validation Standard, 3 vials each, 10 CFU/vial (Mollicutes)

SMB95-2011	Mycoplasma arginini
SMB95-2012	Mycoplasma orale
SMB95-2013	Mycoplasma gallisepticum
SMB95-2014	Mycoplasma pneumoniae
SMB95-2015	Mycoplasma synoviae

SMB95-2016	Mycoplasma fermentans	
SMB95-2017	Mycoplasma hyorhinis	
SMB95-2018	Acholeplasma laidlawii	
SMB95-2019	Spiroplasma citri	
SMB95-2020	Mycoplasma salivarium	

Microsart® Validation Standard, 6 vials each, 99 CFU / vial (bacteria* and fungi)

SMB95-2005	Bacillus subtilis
SMB95-2006	Pseudomonas aeruginosa
SMB95-2007	Kocuria rhizophila
SMB95-2008	Clostridium sporogenes
SMB95-2009	Bacteroides vulgatus
SMB95-2010	Staphylococcus aureus
SMB95-2037	Candida albicans
SMB95-2038	Aspergillus brasiliensis
SMB95-2039	Aspergillus fumigatus
SMB95-2040	Penicillium chrysogenum
SMB95-2041	Candida glabrata
SMB95-2042	Candida krusei
SMB95-2043	Candida tropicalis

* except for Mollicutes

DNA Extraction Kits

SMB95-2001	Microsart® ATMP Extraction (for bacteria and fungi)	50 extractions
SMB95-2003	Microsart® AMP Extraction (for mycoplasma)	50 extractions
56-0002	Proteinase K**	50 extractions

PCR Clean™ **

15-2025	DNA Decontamination Reagent, spray bottle	250 ml
15-2200	DNA Decontamination Reagent, refill bottles	4× 500 ml

PCR Clean™ Wipes **

15-2001	DNA Decontamination Wipes	50 wipes
15-2002	DNA Decontamination Wipes, refill sachets	5× 50 wipes

** Distributed by Minerva Biolabs

Sartorius Stedim Biotech GmbH
August-Spindler-Str. 11
37079 Goettingen, Germany

Phone +49.551.308.0
Fax +49.551.308.3289
www.sartorius-stedim.com

Copyright by
Sartorius Stedim Biotech GmbH,
Goettingen, Germany.
All rights reserved. No part of
this publication may be reprinted
or translated in any form or by any
means without the prior written
permission of
Sartorius Stedim Biotech GmbH.
The status of the information,
specifications and illustrations
in this manual is indicated by
the date given below.
Sartorius Stedim Biotech GmbH
reserves the right to make changes
to the technology, features,
specifications and design of the
equipment without notice.

Status:
November 2019,
Sartorius Stedim Biotech
GmbH, Goettingen, Germany

Printed in Germany on paper that
has been bleached without any use
of chlorine. | W
DIR No.: 2543677-000-00
Ver. 11 | 2019