

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


LOT Lot No.

REF Order No.

 Expiry date

 Store at

 Contains reagents for
100 reactions

 Manufacturer

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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™.
4. Pipettes and gloves should be cleaned thoroughly with PCR Clean™ Wipes prior and during the process.
5. Avoid working above open tubes and avoid air turbulences due to rapid movements.
6. 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	Quantity	Cap Color
	100 Reactions Order No. SMB95-1012	
Fungi Mix	4 × lyophilized	orange
Rehydration Buffer	4 × 0.5 ml	blue
Positive Control DNA	1 × lyophilized	green
Internal Control DNA	4 × lyophilized	yellow
PCR grade Water	5 × 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 (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.

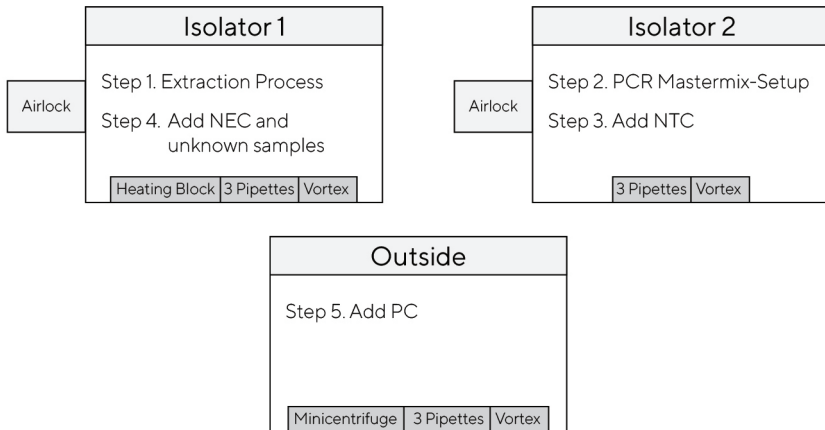
Equipment

- 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
- 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
- Set of 3 pipettes (10 µl, 100 µl and 1000 µl; see previous page for ordering 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

Schematic 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 that DNA extraction prior to PCR is required to achieve highest sensitivity for any kind of sample. While a DNA extraction method providing templates suitable for PCR is available for most types of test samples, several commercially available DNA extraction kits are not DNA- or contaminations-free. For this reason, we recommend combining Microsart® ATMP Fungi with Microsart® ATMP Extraction kit (Prod. No. SMB95-2001): a unique, state-of-the-art DNA extraction method, which eliminates the risk of DNA contaminations and facilitates the detection of fungi and yeast in cell cultures 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 should be prepared. To this aim, we recommend using Microsart® Calibration Reagents as standard material (see Related Products). 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 × Sample	2 × Sample (or extracts)
1 × NEC	1 × NEC
	1 × PC
	1 × NTC
Σ 3 extractions	Σ 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 μ l Rehydration Buffer (blue cap)
3.	Internal Control DNA	yellow cap	Add 800 μ l PCR grade Water (white cap)
4.	Positive Control DNA	green cap	Add 300 μ l 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 μ l including 10 μ l 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 μ l of Internal Control DNA (yellow cap) directly in the Fungi Mix tube (orange cap).
-
2. Vortex and spin briefly.
-
3. Add 15 μ l of the master mix to each PCR tube and close the lid.
Freeze aliquots of any remaining master mix that you might not need for the current assay.
Attention: In order to avoid contaminations, we recommend storing the master mix aliquots in PCR tubes and in a closed and clean rack for PCR.
-

Attention:

If the Internal Control DNA was added to the sample during DNA extraction, add 15 μ l of the Fungi Mix (orange cap) directly to each PCR tube. In this case, do not forget to add 1 μ l of Internal Control DNA to NTC and PC.

8.4 Addition of Samples and Controls

1. Negative controls: add 10 µl Suspension Buffer (violet cap) from the DNA extraction kit or PCR grade Water (white cap). Seal tube tightly before proceeding.
Note: These negative controls should be processed in the isolator/glovebox used for master mix setup.
Important: In order to avoid false positives, do not use the same PCR grade Water tube for NTC and rehydration of the Positive Control DNA.
Important: If the Internal Control DNA was added to the samples during extraction, add 1 µl of Internal Control DNA (yellow cap) also to each negative and positive (step 3.) control.
 2. Sample(s): add 10 µl of sample. Seal tube tightly before proceeding.
Note: Samples and NECs should be added in the isolator/glovebox used for DNA extraction.
 3. Positive controls: add 10 µl Positive Control DNA (green cap). Seal tube tightly.
Attention: Positive controls **should not** be handled in the isolator/glovebox used for master mix setup or DNA extraction.
 4. Spin all PCR tubes briefly.
-

8.5 Start of the qPCR Reaction

1. Load the cycler, check the PCR tubes 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 FAM™ and ROX™ fluorescent signals.
 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. With replicates, take the average of the maximum fluorescence levels. See chapter 10.
 4. Analyze the Ct values calculated for controls and samples.
-

9. Interpretation of Results

The presence of fungal DNA in the sample is indicated by an increasing fluorescence signal in the FAM™ channel during PCR. Based on the obtained Ct values, the unknown concentration of the contaminant can be interpolated along 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 low concentration of Internal Control DNA in the PCR mix, the signal strength in this channel might be reduced with increasing fungal DNA loads in the sample.

9.1 Yes/No Evaluation

Detection of fungi FAM™ channel	Internal Control (IC) ROX™ channel	Interpretation	
positive (Ct < 40)	irrelevant	Fungi/yeast positive	
negative (no Ct)	negative**	if IC used as PCR control	PCR inhibition*
		if IC used as process control	Extract or/and PCR inhibition
negative (no Ct)	positive (Ct < 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, the internal control (ROX™) for fungi negative samples (FAM™: no Ct) must show comparable Ct values to those obtained for the negative controls (NTC) (± 2 cycles). If used as a process control, the internal control (ROX™) for fungi negative samples (FAM™: no Ct) must show comparable Ct values to those obtained for the NTCs (± 3 cycles).

9.2 Total Analysis and recommended Actions for Product Release Testing

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

* Repeat PCR only, if NTC is also positive.

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 performed by an external sequencing service. The interpretation of your sequencing results will be supplied by Minerva Biolabs afterwards.

Attention:

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

10. 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 LightCycler® 480 II (protocol available on request).

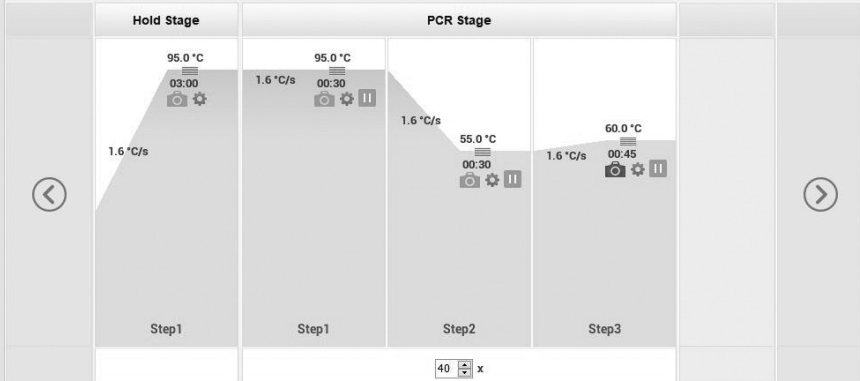
QuantStudio™

- 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.

Experiment Method

Action Save

Volume	Cover
25 μ L	105.0 °C



Legends: Data Collection On Data Collection Off Pause On Pause Off Advanced Settings VeriFlex

Previous

Next

Data Analysis

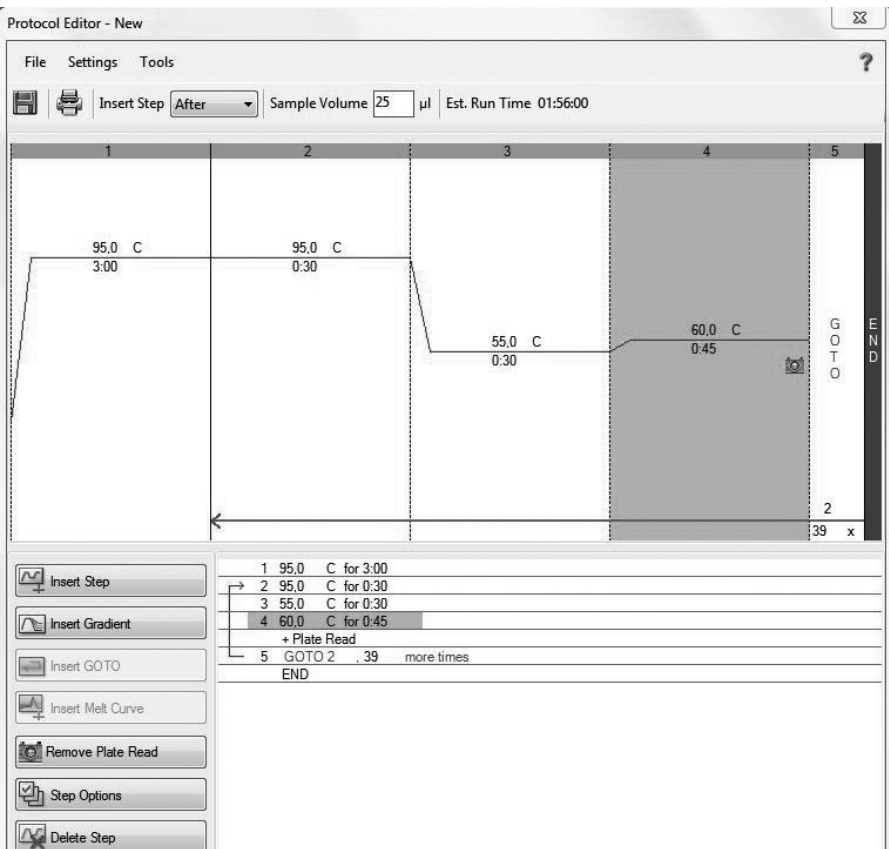
- To enable thresholds setting, please follow the specific instructions provided in the manual of your cycler.
- FAM™: adapt the threshold line to 10 % of the maximum fluorescence level of the positive control. ROX™: adapt the threshold line to 10 % of the maximum fluorescence level of the NTCs. With replicates, 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 on 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
GO TO 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.

The screenshot shows the 'Plate Editor - New' window. The main area displays a 96-well plate grid with columns 1-12 and rows A-H. Each well contains the text 'Unk fungi IC'. The toolbar at the top includes 'File', 'Settings', 'Editing Tools', '100%', 'Scan Mode' (set to 'All Channels'), 'Well Groups...', 'Trace Styles...', 'Spreadsheet View/Importer', and 'Plate Loading Guide'.

On the right side, the 'Select Fluorophores...' panel is active, showing the following settings:

- Sample Type: Unknown
- Load: FAM (Target Name: fungi), ROX (Target Name: IC)
- Load: Sample Name (dropdown: <none>)
- Load: Replicate # (input: 1)

At the bottom, the 'View' section shows 'Plate Type: BR Clear' and several checkboxes: Sample, Well Group, Biological Set, and Well Note. 'OK' and 'Cancel' buttons are located at the bottom right.

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. With replicates, take the average of the maximum fluorescence levels.
- Evaluate the Ct values according to chapter 9.

Rotor-Gene® 6000 (5-plex)

For the use of Rotor-Gene® 6000, 0.1 ml 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 Cyclers:

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

3. 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 performed 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 control. For ROX™: adapt the threshold line to 10 % of the maximum fluorescence level of the NTCs. With replicates, 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

3. 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. With replicates, 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 that 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

- at menu “Run Status” select “Run” and start the cycler by pushing „Start“.

Analysis of raw data:

- In the window **Analysis**, tap on **Analysis Selection / Setup** to analyze 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.
- To set the thresholds manually: 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. With replicates, take the average of the maximum fluorescence levels.
- Read the Ct values in **Text Report**.
- Evaluate the Ct values according to chapter 9.

8. 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
SMB95-1007	Microsart® ATMP Sterile Release	10 samples
SMB95-1008	Microsart® ATMP Bacteria	100 tests
SMB95-1009	Microsart® RESEARCH Bacteria	25 tests
SMB95-1014/1013	Microsart® RESEARCH Fungi	25/100 tests

Microsart® Calibration Reagent, 10⁸ genomes / vial, 1 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, 10⁶ genomes / vial, 1 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, 10 CFU / vial, 3 vials each (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, 99 CFU / vial, 6 vials each (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

SMB95-2001	Microsart® ATMP Extraction (for bacteria and fungi)	50 extractions
SMB95-2003	Microsart® AMP Extraction (for mycoplasma)	50 extractions
SMB95-2002	Microsart® AMP Coating Buffer	20 × 2 ml
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 Reagent, Wipes	50 wipes
15-2002	DNA Decontamination Reagent, refill sachets	5 × 50 wipes

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Notes

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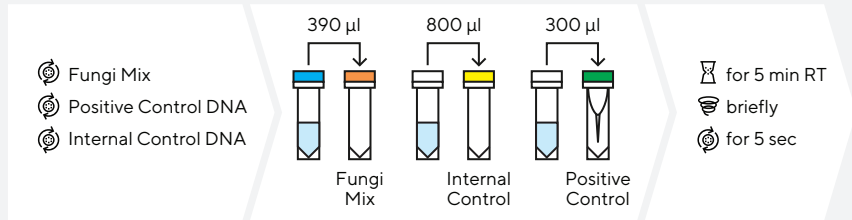
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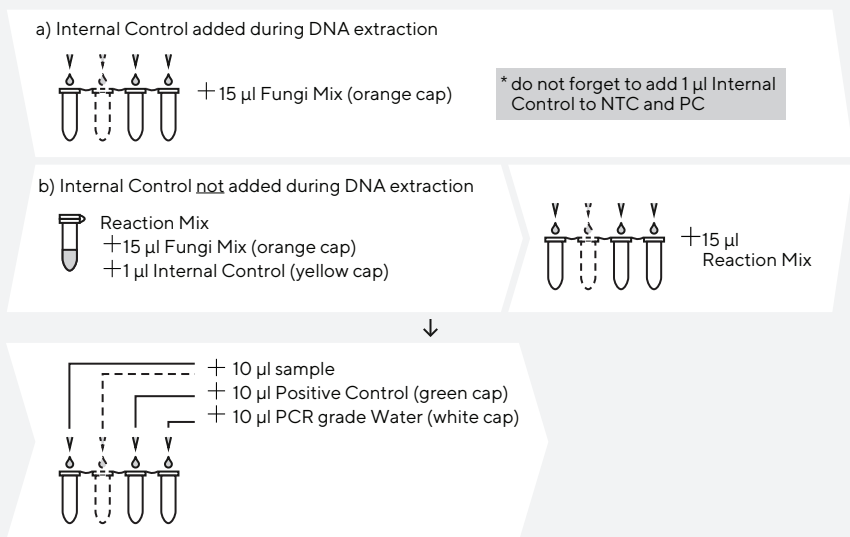
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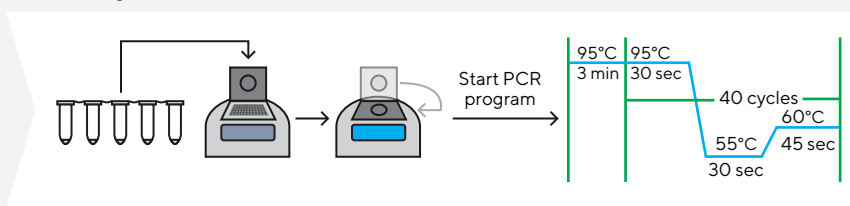
1. Rehydration of Reagents



2. Preparation of PCR Reaction



3. Starting PCR Reaction



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