Instructions for Use

Microsart® RESEARCH Fungi
Fungi and yeast Detection Kit for qPCR

Prod. No. SMB95-1013 | SMB95-1014
Reagents for 100 | 25 reactions
For use in research

Manufactured by:

Minerva Biolabs GmbH
Schkopauer Ring 13
12681 Berlin
Germany
Symbols

**LOT** Lot No.

**REF** Order No.

 срок годности: Expiry date

хранить при температуре: Store at

содержит реагенты для: Contains reagents for 25 or 100 tests

производитель: Manufacturer
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1. Intended Use

Microsart® RESEARCH Fungi is used for direct detection of fungal contamination in cell cultures and cell media components in research and development.

2. Explanation of the Test

Microsart® RESEARCH Fungi utilizes real-time PCR (qPCR). The assay can be performed with any type of real-time PCR cycler able to detect the fluorescent dyes FAM™ and ROX™. The provided protocol is applied preferably for fast and reliable screenings of cell culture supernatants, mostly required in research and development. The detection procedure can be performed within three hours. In contrast to the culture method, samples do not need to contain living fungi.

3. Test Principle

Fungi are specifically detected by amplifying a highly conserved rRNA operon, or more specifically, a 18S rRNA coding region of the fungal genome. The 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 described so far as contaminants of cell cultures and media components. Bacterial 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, included in the Fungi RESEARCH Mix. 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. 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.

4. Always use a new unopened DNA-free pipette filter tip-box for each assay. Reaction vials should always be closed immediately after every pipetting step.

5. It is recommended to perform the assay in a predecontaminated, UV-treated laminar flow cabinet. Spatial segregation of the sequential steps is recommended.

6. In case of working with living fungi strains, the local regulatory requirements for S2 labs must be considered.

7. Attention: by aliquoting and freezing your samples you run a high risk of contamination. This should therefore be avoided if possible.

8. This leaflet must be widely understood for a successful use of Microsart® RESEARCH 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.

9. Any deviation from the test method can affect the results.

10. 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.

11. 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.
12. 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.

13. 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
1. The clean bench should be cleaned thoroughly with PCR Clean™ (Minerva Biolabs, Prod. No. 15-2025) or PCR Clean™ Wipes (Minerva Biolabs, Prod. No. 15-2001) before use.

2. All materials which are introduced into the clean bench should be cleaned thoroughly with PCR Clean™ prior the process.

3. Avoid working above open tubes and avoid air turbulences due to rapid movements.

4. Be careful when opening the tubes. Do not touch the inner surface of the lid.
5. Reagents

Each kit contains reagents for 25 or 100 reactions. The expiry date of the unopened package is marked on the package label. The kit components are stored until use at +2 to +8 °C and must be stored at ≤ -18 °C after rehydration. Protect the Fungi RESEARCH Mix from light.

<table>
<thead>
<tr>
<th>Kit Component Label Information</th>
<th>Quantity</th>
<th>Cap Color</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fungi RESEARCH Mix</strong></td>
<td>1 × lyophilized</td>
<td>4 × lyophilized</td>
</tr>
<tr>
<td><strong>Rehydration Buffer</strong></td>
<td>1 × 1.0 ml</td>
<td>4 × 1.0 ml</td>
</tr>
<tr>
<td><strong>Positive Control DNA</strong></td>
<td>1 × lyophilized</td>
<td>1 × lyophilized</td>
</tr>
<tr>
<td><strong>PCR Grade Water</strong></td>
<td>1 × 1.5 ml</td>
<td>1 × 1.5 ml</td>
</tr>
</tbody>
</table>

The lot specific Certificate of Analysis can be downloaded from the manufacturer's website (www.minerva-biolabs.com).
6. Needed but not Included

Microsart® RESEARCH Fungi contains the reagents for the specific detection of fungi. 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 fungal 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)
- **Optional**: Microsart® ATMP Extraction kit, a DNA-free extraction kit, Sartorius Prod. No. SMB95-2001.

**Equipment**
- 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 2 ml reaction tubes and PCR-tubes
- Vortex mixer
- 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
- **Optional**: heat block preferably with shaking function, when performing DNA extraction prior to PCR.
7. Specimen

Microsart® RESEARCH Fungi does not require DNA extraction prior to use. Samples can be obtained directly from cell culture supernatants. Samples directly received from cell cultures contain DNases which can degrade fungal DNA even at lower temperatures. If the test cannot be performed immediately after sample collection, samples should be stabilized by heat inactivation at 95 °C for 10 min and stored at \( \leq -18 \) °C until use.

1. Transfer up to 500 μl of cell culture supernatant or cell culture material with max.\( 10^6 \) cells/ml into a sterile microcentrifuge tube. The lid should be tightly sealed to prevent opening during heating.
2. Incubate the sample at 95 °C for 10 minutes.
3. Briefly centrifuge (5 seconds) the sample at approx. 13,000 x g to pellet cellular debris.
4. The supernatant is used for PCR analysis.

If you detect any inhibitory effects, DNA extraction i.e. with Microsart® ATMP Extraction (Prod. No. SMB95-2001) is mandatory. 2 μl of the extract can be used directly as PCR template.

Repeated freezing and thawing of samples should be avoided.
8. Test Procedure

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

8.1 Rehydration of the reagents

After reconstitution, the reagents should be stored at ≤ -18 °C. Repeated freezing and thawing should be avoided and the reconstituted Positive Control must be stored in aliquots.

<table>
<thead>
<tr>
<th>Step</th>
<th>Fungi RESEARCH Mix</th>
<th>Positive Control DNA</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>orange cap</td>
<td>green cap</td>
<td>Spin all lyophilized components for 5 sec at maximum speed of the microcentrifuge.</td>
</tr>
<tr>
<td>2</td>
<td>orange cap</td>
<td>green cap</td>
<td>Add 600 μl Rehydration Buffer (blue cap). Add 300 μl PCR grade water (white cap).</td>
</tr>
<tr>
<td>3</td>
<td>orange cap</td>
<td>green cap</td>
<td>Incubate 5 min at room temperature.</td>
</tr>
<tr>
<td>4</td>
<td>orange cap</td>
<td>green cap</td>
<td>Vortex briefly</td>
</tr>
</tbody>
</table>
8.2 Loading the test tubes

This process 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.

1. Homogenize the rehydrated Fungi RESEARCH Mix by vortexing. Spin for 5 sec.
2. Add 23 μl to each PCR tube.
3. Negative control: Add 2 μl PCR grade Water (white cap)
4. Sample reaction: Add 2 μl of sample.
5. Positive control: Add 2 μl Positive Control DNA (green cap).
6. Close and spin all PCR tubes briefly, load the qPCR cycler and start the program.

8.3 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.

8.4 Analysis

1. Save the data at the end of the run.
2. Show amplification plots for FAM™ and ROX™ in linear mode.
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. Read the calculation of the Ct-values for the negative controls, the positive controls and the samples.
9. Short Instructions

1. Rehydration of Reagents

- Load 23 µl Fungi RESEARCH Mix into the test tubes.
- Add 600 µl Fungi RESEARCH Mix and 300 µl Positive Control.
- Incubate briefly for 5 sec.

2. Preparation of PCR Reactions

- Add 2 µl sample loading the test tubes.
- Add 2 µl Positive Control to the reaction mixture.
- Add 2 µl PCR Grade Water (negative control).
- Vortex and centrifuge the samples.

3. Starting the PCR Reaction

- Start PCR program:
  - 95°C for 3 min
  - 45 sec cycles
  - 55°C for 30 sec
  - 60°C for 45 sec

- 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 Ct 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 the internal control channel (ROX™). 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 fungi DNA loads in the sample.

10.1 Yes/No Evaluation

<table>
<thead>
<tr>
<th>Detection of fungi FAM™ channel</th>
<th>Internal Control ROX™ channel</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>positive (Ct &lt; 40)</td>
<td>irrelevant</td>
<td>Fungi positive</td>
</tr>
<tr>
<td>negative (no Ct)</td>
<td>negative **</td>
<td>PCR inhibition*</td>
</tr>
<tr>
<td>negative (no Ct)</td>
<td>positive (Ct &lt; 40)</td>
<td>Fungi negative</td>
</tr>
</tbody>
</table>

* 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.

** Internal control of fungi negative samples (FAM™: no Ct) must show Ct-values in the range of +/- 2 cycles (ROX™) of the no-template control (master mix control).
### 10.2 Total Analysis and recommended actions

<table>
<thead>
<tr>
<th>Sample</th>
<th>Result</th>
<th>Interpretation</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTC</td>
<td>negative</td>
<td>Valid PCR</td>
<td>Interpret specimen results</td>
</tr>
<tr>
<td></td>
<td>positive</td>
<td>PCR contamination</td>
<td>Repeat the test</td>
</tr>
<tr>
<td>PC</td>
<td>positive</td>
<td>Valid PCR</td>
<td>Interpret specimen results</td>
</tr>
<tr>
<td></td>
<td>negative</td>
<td>Failed PCR</td>
<td>Repeat the test</td>
</tr>
<tr>
<td>Specimen</td>
<td>0/2 positive</td>
<td>No contamination</td>
<td>Interpret result as negative</td>
</tr>
<tr>
<td></td>
<td>1/2 positive</td>
<td>Possible contamination</td>
<td>Repeat the test. If result is confirmed, interpret as positive</td>
</tr>
<tr>
<td></td>
<td>2/2 positive</td>
<td>Contamination</td>
<td>Interpret result as positive</td>
</tr>
</tbody>
</table>

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 successfully tested with the Microsart® RESEARCH Fungi kit:

QuantStudio™, Mx3005P™, CFX96 Touch™, CFX96 Touch Deep Wellc™, LightCycler®
480 II (available on request), ABI Prism® 7500, Rotor-Gene® Q/Rotor-Gene®

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 to 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
- View amplification curves of FAM™ channel by selecting the FAM™ checkbox under the amplification plot
- 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, 0.1 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:

<table>
<thead>
<tr>
<th>Target</th>
<th>Fungi</th>
<th>Internal Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filter</td>
<td>green</td>
<td>orange</td>
</tr>
<tr>
<td>Wavelength</td>
<td>470–510 nm</td>
<td>585-610 nm</td>
</tr>
</tbody>
</table>

2. Program the Cycler:

**Program 1: Pre-incubation**

<table>
<thead>
<tr>
<th>Setting</th>
<th>Hold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hold Temperature</td>
<td>95 °C</td>
</tr>
<tr>
<td>Hold Time</td>
<td>3 min 0 sec</td>
</tr>
</tbody>
</table>

**Program Step 2: Amplification**

<table>
<thead>
<tr>
<th>Setting</th>
<th>Cycling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycles</td>
<td>40</td>
</tr>
<tr>
<td>Denaturation</td>
<td>95 °C for 30 sec</td>
</tr>
<tr>
<td>Annealing</td>
<td>55 °C for 30 sec</td>
</tr>
<tr>
<td>Elongation</td>
<td>60 °C for 45 sec —&gt; acquiring to Cycling A (green and orange)</td>
</tr>
<tr>
<td>Gain setting</td>
<td>automatic (Auto-Gain)</td>
</tr>
<tr>
<td>Slope Correct</td>
<td>activated</td>
</tr>
<tr>
<td>Ignore First</td>
<td>deactivated</td>
</tr>
</tbody>
</table>
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. 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:

<table>
<thead>
<tr>
<th>Target</th>
<th>Fungi</th>
<th>Internal Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filter</td>
<td>FAM™</td>
<td>ROX™</td>
</tr>
<tr>
<td>Wavelength</td>
<td>470-510 nm</td>
<td>585-610 nm</td>
</tr>
<tr>
<td>Quencher</td>
<td>none</td>
<td>none</td>
</tr>
</tbody>
</table>

**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**

<table>
<thead>
<tr>
<th>Setting</th>
<th>Hold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>95 °C</td>
</tr>
<tr>
<td>Incubation time</td>
<td>3 min</td>
</tr>
</tbody>
</table>

**Program Step 2: Amplification**

<table>
<thead>
<tr>
<th>Cycles</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Setting</td>
<td>Cycle</td>
</tr>
<tr>
<td>Denaturing</td>
<td>95 °C for 30 sec</td>
</tr>
<tr>
<td>Annealing</td>
<td>55 °C for 30 sec</td>
</tr>
<tr>
<td>Extension</td>
<td>60 °C for 45 sec</td>
</tr>
</tbody>
</table>
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.
  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:
- 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" for an automatic threshold.
- Read the Ct-values in "Text Report"
- 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 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, Inc. 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 GmbH. PCR Clean is a trademark of Minerva Biolabs GmbH.

Last technical revision: 2019-11-15
## 12. Related Products

### Detection Kits for qPCR

<table>
<thead>
<tr>
<th>Code</th>
<th>Product Description</th>
<th>Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMB95-1001/1002</td>
<td>Microsart® AMP Mycoplasma</td>
<td>25/100 tests</td>
</tr>
<tr>
<td>SMB95-1003/1004</td>
<td>Microsart® ATMP Mycoplasma</td>
<td>25/100 tests</td>
</tr>
<tr>
<td>SMB95-1005/1006</td>
<td>Microsart® RESEARCH Mycoplasma</td>
<td>25/100 tests</td>
</tr>
<tr>
<td>SMB95-1007</td>
<td>Microsart® ATMP Sterile Release</td>
<td>10 samples</td>
</tr>
<tr>
<td>SMB95-1008</td>
<td>Microsart® ATMP Bacteria</td>
<td>100 tests</td>
</tr>
<tr>
<td>SMB95-1009</td>
<td>Microsart® RESEARCH Bacteria</td>
<td>25 tests</td>
</tr>
<tr>
<td>SMB95-1012</td>
<td>Microsart® ATMP Fungi</td>
<td>100 tests</td>
</tr>
</tbody>
</table>

### Microsart® Calibration Reagent, 1 vial, 10⁸ genomes / vial (bacteria, including Mollicutes)

<table>
<thead>
<tr>
<th>Code</th>
<th>Product Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMB95-2021</td>
<td>Mycoplasma arginini</td>
</tr>
<tr>
<td>SMB95-2022</td>
<td>Mycoplasma orale</td>
</tr>
<tr>
<td>SMB95-2023</td>
<td>Mycoplasma gallisepticum</td>
</tr>
<tr>
<td>SMB95-2024</td>
<td>Mycoplasma pneumoniae</td>
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<tr>
<td>SMB95-2025</td>
<td>Mycoplasma synoviae</td>
</tr>
<tr>
<td>SMB95-2026</td>
<td>Mycoplasma fermentans</td>
</tr>
<tr>
<td>SMB95-2027</td>
<td>Mycoplasma hyorhinis</td>
</tr>
<tr>
<td>SMB95-2028</td>
<td>Acholeplasma laidlawii</td>
</tr>
<tr>
<td>SMB95-2029</td>
<td>Spiroplasma citri</td>
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<tr>
<td>SMB95-2030</td>
<td>Bacillus subtilis</td>
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<tr>
<td>SMB95-2031</td>
<td>Pseudomonas aeruginosa</td>
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<tr>
<td>SMB95-2032</td>
<td>Kocuria rhizophila</td>
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<tr>
<td>SMB95-2033</td>
<td>Clostridium sporogenes</td>
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<tr>
<td>SMB95-2034</td>
<td>Bacteroides vulgatus</td>
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<tr>
<td>SMB95-2035</td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td>SMB95-2036</td>
<td>Mycoplasma salivarium</td>
</tr>
</tbody>
</table>

### Microsart® Calibration Reagent, 1 vial, 10⁶ genomes / vial (fungi)

<table>
<thead>
<tr>
<th>Code</th>
<th>Product Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMB95-2044</td>
<td>Candida albicans</td>
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<tr>
<td>SMB95-2045</td>
<td>Aspergillus brasiliensis</td>
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<tr>
<td>SMB95-2046</td>
<td>Aspergillus fumigatus</td>
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<tr>
<td>SMB95-2047</td>
<td>Penicillium chrysogenum</td>
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<tr>
<td>SMB95-2048</td>
<td>Candida glabrata</td>
</tr>
<tr>
<td>SMB95-2049</td>
<td>Candida krusei</td>
</tr>
<tr>
<td>SMB95-2050</td>
<td>Candida tropicalis</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Code</th>
<th>Product Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMB95-2011</td>
<td>Mycoplasma arginini</td>
</tr>
<tr>
<td>SMB95-2012</td>
<td>Mycoplasma orale</td>
</tr>
<tr>
<td>SMB95-2013</td>
<td>Mycoplasma gallisepticum</td>
</tr>
<tr>
<td>SMB95-2014</td>
<td>Mycoplasma pneumoniae</td>
</tr>
</tbody>
</table>
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 x 500 ml

**PCR Clean™ Wipes**
15-2001  DNA Decontamination Wipes  50 wipes
15-2002  DNA Decontamination Wipes, refill sachets  5 x 50 wipes

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