

Microsart® ATMP Sterile Release

Bacteria, fungi, and yeasts DNA extraction and qPCR detection kit
Prod. No. SMB95-1007

Reagents for 10 samples
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
25 or 100 reactions



Manufacturer

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1. Intended Use

Microsart® ATMP Sterile Release kit is designed for DNA extraction of bacteria, fungi, and yeasts in cell culture derived biologicals, like Advanced Therapy Medicinal Products (ATMPs), and for direct detection based on real-time PCR (qPCR). Be aware that this product is not intended to be used as a diagnostic kit.

2. Explanation of the Test

Microsart® ATMP Sterile Release utilizes qPCR as the method of choice for sensitive and robust detection of bacterial or fungal contamination. To achieve highest sensitivity and avoid inhibitory effects in PCR testing, the DNA is extracted prior to PCR.

Microsart® ATMP Sterile Release introduces a unique DNA extraction method, which reduces the risk of DNA contaminations, facilitating the detection of contaminants in cell culture and ATMPs via PCR. The subsequent qPCR assay can be performed with virtually any type of real-time PCR cycler able to detect the fluorescent dyes FAM™ and ROX™. The complete detection procedure can be performed within 3.5 hours. In contrast to the culture method, samples do not need to contain living material as all intact particles (e.g. live, dormant, non-culturable etc.) are detected.

3. Test Principle

Microsart® ATMP Sterile Release kit was optimized for the extraction and detection of genomic bacterial and fungal DNA in cell culture samples. The contamination risk has been minimized due to the reduced number of handling steps.

Bacteria are specifically detected by amplifying a highly conserved region of the rRNA operon, or more specifically, a fragment of the 16S rRNA coding region in the bacterial genome while fungi and yeasts are specifically detected by amplifying a fragment of the highly conserved 18S rRNA coding region.

Each specific amplification is detected at 520 nm (FAM™ channel). The kit includes primer and FAM™ labeled probes, which allow the specific detection of many bacterial and fungal species. Both master mixes contain the polymerase. False negative results due to PCR inhibitors or improper DNA extraction are detected by using the internal amplification control. 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 leaflet must be widely understood for a successful use of Microsart® ATMP Sterile Release. 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.
3. This kit should be used by trained staff, only. A clean lab coat and disposable gloves should be worn at all times while performing the assay.
4. To avoid DNA cross-contaminations, the complete test must be performed under sterile and DNA-free conditions (see chapter 4.1 for detailed information).
5. In case of work with living strains, the local regulation for S2 laboratories must be followed.
6. This detection kit has been developed for 1 ml starting volume. When using less than 1 ml, please make sure that 99 cfu can be detected in the selected volume.
7. This kit is not validated for the extraction of mycoplasma DNA.
8. Any deviation from the test method can affect the results.
9. For each test setup, at least one negative extraction control and at least one PCR negative control should be included. Positive controls facilitate the evaluation of the test. Typical Ct values for the internal control and PCR positive control are shown on the Certificate of Analysis and can be used as a guideline for quality control.
10. Controls should be handled in the same manner as the samples.
11. Assay inhibition may be caused by the sample matrix but also by sample elution buffers of incompatible or non-validated DNA extraction methods. Do not use reagents from another kit than the Microsart® ATMP Sterile Release.
12. 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 perform the test under sterile and DNA-free conditions, we recommend the use of an isolator/glovebox with an airlock.
2. The isolator/glovebox should be thoroughly cleaned with PCR Clean™ (Minerva Biolabs, Prod. No. 15-2025) or PCR Clean™ Wipes (Minerva Biolabs, Prod. No. 15-2001) before and during the work process.
3. All materials that are introduced in the isolator/glovebox should be cleaned thoroughly with PCR Clean™. Do not forget to clean the airlock with PCR Clean™. Pipettes and gloves should be thoroughly cleaned 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 all reagents needed to test 10 samples. It consists of 10 individual sample tests containing material for three DNA extractions, five bacteria PCR reactions, and five fungi PCR reactions. The expiry date of the unopened package is marked on the package label. The kit components are stored at +2 – +8 °C until use. Protect the SR Mixes from light.

Kit Component Label Information	10 samples Order No. SMB95-1007	Cap Color
Lysis Buffer	10 x 1.8 ml	transparent
Suspension Buffer	10 x 0.4 ml	violet
Processing Tubes	10 x 3	–
Bacteria SR Mix	10 x lyophilized	red
Fungi SR Mix	10 x lyophilized	orange
Rehydration Buffer	10 x 0.3 ml	blue
Positive Control DNA	10 x lyophilized	green
Internal Control DNA	10 x lyophilized	yellow
PCR grade Water	20 x 0.3 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

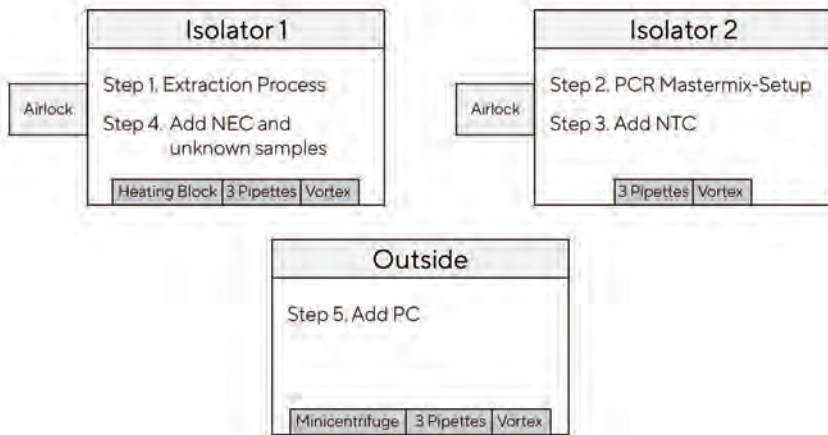
Microsart® ATMP Sterile Release kit contains reagents for DNA extraction and DNA detection. General industrial supplies and reagents, usually available in PCR laboratories are not included:

Consumables

- Laboratory gloves
- PCR Clean™ (Minerva Biolabs, Prod. No. 15-2025) and PCR Clean™ wipes (Minerva Biolabs, Prod. No. 15-2001)
- DNA-free pipette filter tips that must be free from bacterial and 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)
- 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)

Equipment

- Isolator/glovebox (further information, supplier and prices are available on request, please contact PCR@sartorius.com)
- Heat block with optional shaking function
- Microcentrifuge for 1.5 ml reaction tubes (Centrisart A-14, Prod. No. A-14-1EU)
- Vortex
- 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 PCR tubes
- 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
- Rack for 1.5 ml tubes and for PCR-tube strips



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. Test Procedure

7.1 Recommendation for product release testing

Each sample should be extracted in duplicate along with a negative extraction control (NEC) (= 3 extractions for 1 sample/product). Each obtained extract should be loaded 1 x for the bacteria and 1 x for the fungi qPCR assay. Additionally, each PCR assay should include 1 x PCR negative control (no-template control, NTC) and 1 x PCR positive control (PC).

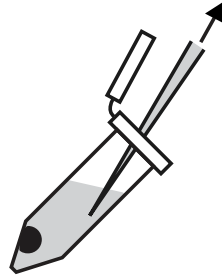
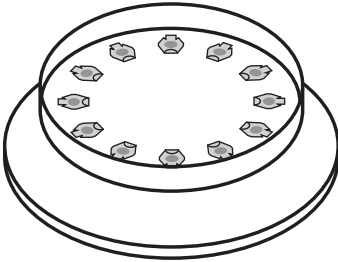
DNA extraction	PCR with Bacteria SR Mix	PCR with Fungi SR Mix
2 x Sample	2 x Sample Extracts	2 x Sample Extracts
1 x NEC	1 x NEC	1 x NEC
	1 x PC	1 x PC
	1 x NTC	1 x NTC
Σ 3 extractions	Σ 5 PCR reactions	Σ 5 PCR reactions

7.2 Sample Collection and Storage

The kit has been validated using a maximum cell concentration of 10^6 cells/ml. Notably, the assay can be performed with different types of cell culture-derived material. Therefore, the optimal sampling parameters, like volume or cell number, can vary according to the specific characteristics of the sample (e.g. medium, cell type) and may require optimization of the procedure.

1. max. 1 ml of cell culture or cell culture supernatant liquid material is transferred into a provided DNA-free 1.5 ml processing tube (transparent cap).
2. Spin down for 15 minutes at a speed of at least 16,200 x g to sediment particles. **Attention:** Make sure to position the tubes in the centrifuge in order to obtain a pellet on the back side of the tube, as described in the figure below.

-
3. Discard the supernatant carefully and completely as described in the figure below. Proceed to DNA extraction. If DNA extraction cannot be performed immediately, freeze samples at $\leq -18\text{ }^{\circ}\text{C}$. Repeated freezing and thawing should be avoided.
- Attention:** Samples can only be inactivated or frozen after this sample collection step.
-



Make sure to position the tubes with the back side toward the outside of the rotor in order to obtain a pellet on the back wall of the tube.

Slowly discard all the supernatant without disturbing the pellet

7.3 DNA Extraction Process

1. Add 500 μ l Lysis Buffer (transparent cap) to cell pellet.
Optional: The Internal Control DNA can also be used to monitor the extraction process. Add 20 μ l Internal Control DNA to the sample, vortex briefly and proceed with step 2 as described.
No additional Internal Control DNA is required for the PCR reaction mix.

2. Vortex vigorously for at least 30 seconds until pellet is completely dissolved.

3. Heat at 80 °C (optional with shaking) for 10 minutes.

4. Spin down at 16,200 x g for 10 minutes
Attention: Make sure to position the tubes in the rotor as indicated in the figure in chapter 7.2.

5. Remove supernatant carefully and completely, following the explanations in chapter 7.2. Make sure not to disturb or aspirate the pellet in the process.
Attention: There is a higher risk of inhibition in PCR analysis if residues remain in the tube.

6. Add 100 μ l Suspension Buffer (violet cap) and dissolve the DNA by thorough vortexing.

Extracts can be stored for 6 days at +2 to +8 °C. If long term storage is required, store at \leq -18 °C. Repeated freezing and thawing should be avoided.

7.4 Rehydration of the Reagents

1.	Bacteria SR Mix Fungi SR Mix Internal Control DNA Positive Control	red cap orange cap yellow cap green cap	Centrifuge briefly
2.	Bacteria SR Mix Fungi SR Mix	red cap orange cap	Add 90 µl Rehydration Buffer (blue cap)
3.	Internal Control DNA	yellow cap	Add 100 µl PCR grade Water (white cap)
4.	Positive Control DNA	green cap	Add 100 µl PCR grade Water (white cap)
5.	Bacteria SR Mix Fungi SR Mix Internal Control DNA Positive Control DNA	red cap orange cap yellow cap green cap	Incubate 5 min at room temperature
6.	Bacteria SR Mix Fungi SR Mix Internal Control DNA Positive Control DNA	red cap orange cap yellow cap green cap	Vortex briefly

7.5 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.

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 6 μ l of Internal Control DNA (yellow cap) to each rehydrated Mix (red cap for Bacteria SR Mix; orange cap for Fungi SR Mix).
 2. Homogenize the reaction mix by tapping carefully against the tube. Spin briefly.
 3. Add 15 μ l to each PCR tube. Close PCR tubes. Discard remaining liquid.
-

Attention:

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

7.6 Loading of the Test Tubes

1. Negative controls: add 10 µl Suspension Buffer (violet cap) or PCR grade Water (white cap). Seal tube before proceeding with the samples.

Attention: Negative controls should be processed in the isolator/glovebox used for master mix setup.

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.

Important: If the Internal Control DNA was added to the samples during DNA extraction, add 1 µl of Internal Control DNA to each negative and positive control.

4. Close and spin all PCR tubes briefly, load the qPCR cyclers and start the program.
-

7.7 Start of 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.
-

7.8 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 10.
-

4. Analyze the calculation of the Ct values for negative controls, positive controls and samples.
-

8. Interpretation of Results

The presence of 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 correspondence of the internal control channel. Target 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 contaminant DNA loads in the sample.

8.1 Yes/No Evaluation

Detection of contaminants: FAM™ channel	Internal Control: ROX™ channel	Interpretation	
positive (Ct < 40)	irrelevant	Target DNA positive	
negative (no Ct)	negative**	if used as PCR control	PCR inhibition*
		if used as process control	Extraction or/and PCR inhibition
negative (no Ct)	positive (Ct < 40)	Target DNA negative	

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

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

8.2 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.

Use these analytical guidelines for each set of obtained results, namely for bacteria or fungi contaminations, separately.

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.

9. Appendix

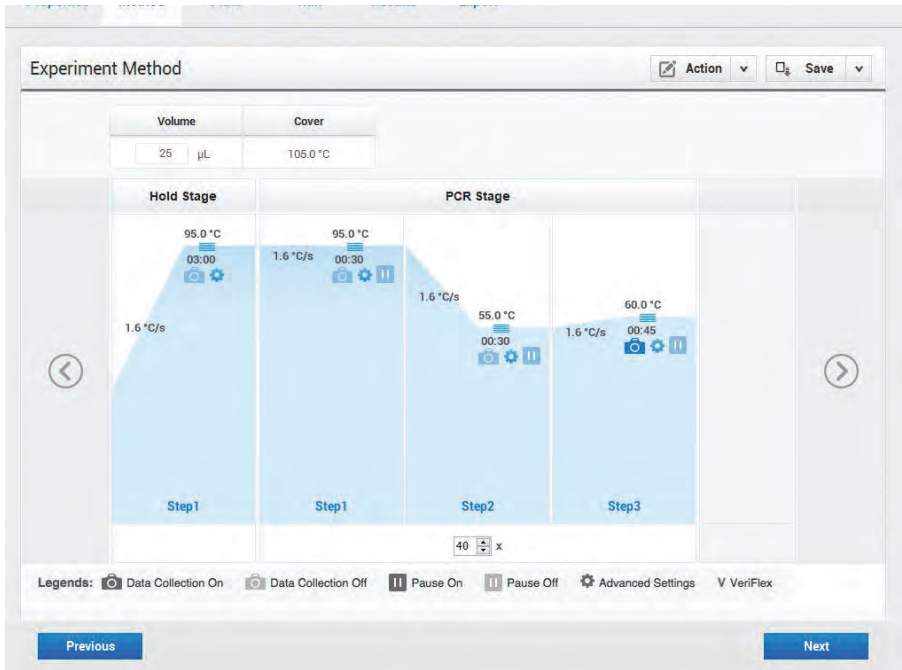
The protocol can be performed with any type of real-time PCR cycler able to detect the fluorescent dyes FAM™ and ROX™.

The following qPCR cyclers were used for the validation of Microsart® Sterile Release: QuantStudio™, Mx3005P™, CFX96 Touch™, CFX96 Touch Deep Well™, ABI Prism® 7500, Rotor-Gene® Q/Rotor-Gene®.

In addition, Microsart® ATMP Sterile Release kit was successfully tested with the Light-Cycler® 480 II. A detailed protocol is 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

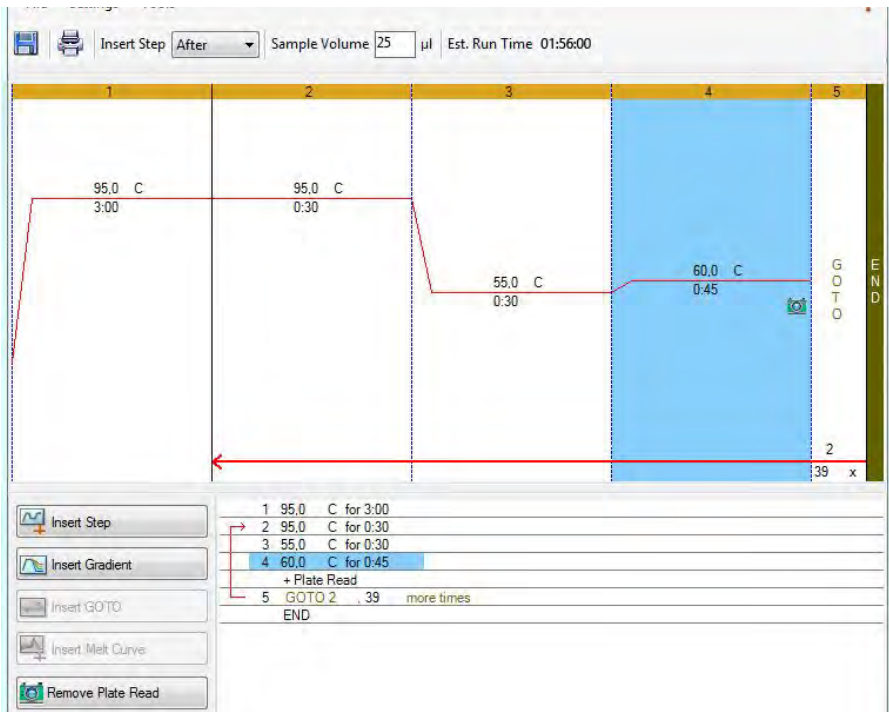
- To enable thresholds setting, please follow the specific instructions provided in the manual of your cycler.
- FAM™: adapt the threshold line to 10 % of the maximum fluorescence level of the positive control. ROX™: adapt the threshold line to 10 % of the maximum fluorescence level of the NTCs. In case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

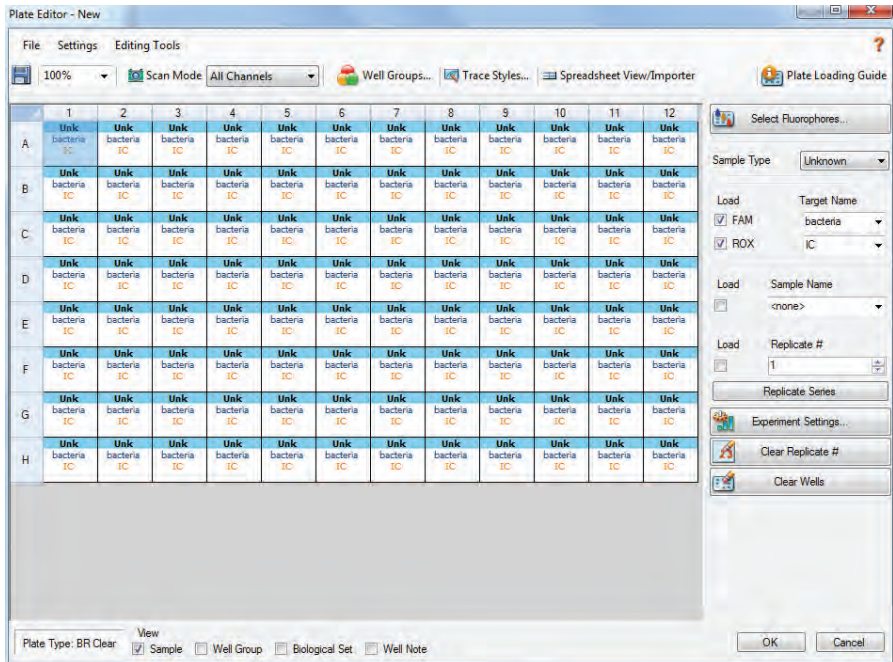
- Click File -> New -> Protocol to open the Protocol Editor and create a new protocol
- Select any step in either the graphical or text display
- Click the temperature or well time to directly edit the value

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 with "Sample Type"
- Name your samples with "Sample Type"
- 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 target DNA 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 check-boxes located under amplification chart. Select FAM™ to display data of bacteria or funghi 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 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.
- 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	Bacteria or Fungi/Yeasts	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

Analysis:

Please analyze the results of the bacteria and fungi assays, separately.

- Open the menu Analysis
- Select Quantitation
- Check the required filter set (green and orange) according to the table above 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 included in the run 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	Bacteria or Fungi/Yeasts	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:

- Please analyze the results of the bacteria and fungi assays, separately.
- 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 cyclor by pushing „Start“

Analysis of raw data:

- Please analyze the results of the bacteria and fungi assays, separately.
- In the window "Analysis" tab 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.
- 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 9.

10. 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
SMB95-1008	Microsart® ATMP Bacteria	100 tests
SMB95-1012	Microsart® ATMP Fungi	100 tests
SMB95-1014/1013	Microsart® RESEARCH Fungi	25/100 tests

Microsart® Calibration Reagent, 1 vial, 10⁸ 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, 10⁶ 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 Kit

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

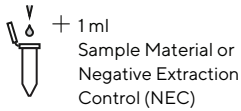
PCR Clean™ Wipes**

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

** Distributed by Minerva Biolabs

Short Instructions

1. Sample Collection



Processing tubes

15 min
≥ 16,200 × g



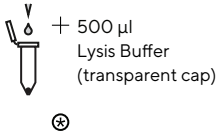
Discard
supernatant

Store at ≤ -18 °C



or Proceed to
DNA Extraction

2. DNA Extraction

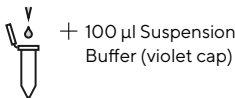


⊛

≥ 30 sec vigorously
80 °C, 10 min
≥ 16,200 × g, 10 min



Remove supernatant
carefully



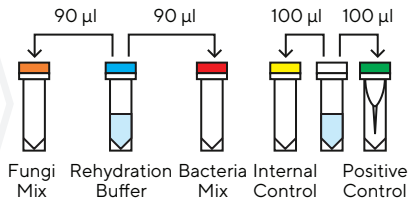
≥ 30 sec vigorously



DNA ready for PCR

3. Rehydration of Reagents

- ⊛ Bacteria SR Mix
- ⊛ Fungi SR Mix
- ⊛ Positive Control DNA
- ⊛ Internal Control DNA



⌚ for 5 min RT
🌀 briefly
⊛ for 5 sec

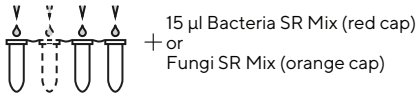
- ⌚ incubate
- 🌀 vortex
- ⊛ centrifuge
- + add

⊛ Optional:
Add Internal Control from Microsart® ATMP Sterile Release

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

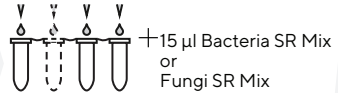
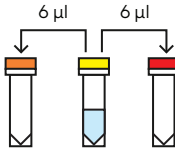
4. Preparation of PCR Reaction Mix

a) Internal Control added during DNA extraction

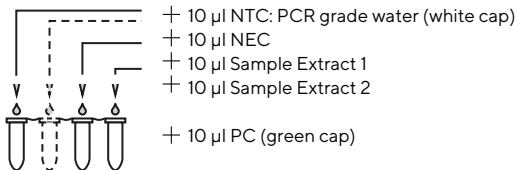


* do not forget to add 1 µl Internal Control to NTC and PC

b) Internal Control not added during DNA extraction

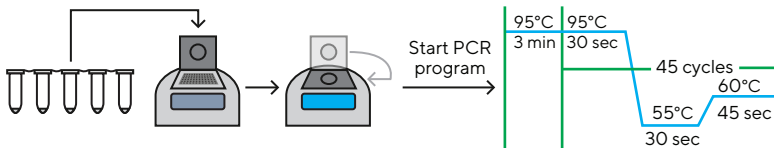


5. Addition of Samples and Controls



Close lid tightly
⊗ briefly

6. Start of the qPCR Reaction



- Rehydration Buffer
- Mycoplasma Mix
- PCR grade Water
- Positive Control
- Internal Control

- incubate
- vortex
- centrifuge
- add

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

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Notes

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