Instructions for Use

Microsart® RESEARCH Bacteria

Bacteria Detection Kit for qPCR

Prod. No. SMB95-1009 | SMB95-1010

Reagents for 25 |100 reactions

For use in research

Manufactured by:

Minerva Biolabs GmbH
Koepenicker Strasse 325
12555 Berlin
Germany

Symbols

LOT	**Lot No.**
REF	**Order No.**
	**Expiry date**
	**Store at**
	**Contains reagents for 25 or 100 tests**
	**Manufacturer**

Contents

1. Intended Use **5**

2. Explanation of the Test **5**

3. Test Principle **5**

4. Precautions **6**

5. Reagents **7**

6. Needed but not Included **8**

7. Specimen **9**

8. Test Procedure **10**
8.1 Rehydration of the reagents 10
8.2 Loading the test tubes 11
8.3 Starting the reaction 11
8.4 Analysis 11

9. Short Instructions **12**

10. Notes on the Test Procedure **13**

11. Interpretation of Results **14**

12. Appendix **15**

13. Related Products **22**

1. Intended Use

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

2. Explanation of the Test

Microsart® RESEARCH Bacteria utilizes real-time PCR (qPCR). The assay can be performed with any type of real-time PCR cycler able to detect the fluorescence dyes FAM™ and ROX™. The protocol provided is preferred for fast and reliable screening of cell culture supernatants most applicable in research and development. The detection procedure can be performed within 3 hours. In contrast to the detection by culture, samples do not need to contain vital bacteria.

3. Test Principle

Bacteria are specifically detected by amplifying a highly conserved rRNA operon, or more specifically, a 16S rRNA coding region in the bacterial genome. The amplification is detected at 520 nm (FAM™ channel). The kit includes primer and FAM™ labeled probes which allow the specific detection of many bacterial species so far described as contaminants of cell cultures and media components. Eukaryotic DNA is not amplified by this primer/probe system.

False negative results due to PCR inhibitors or improper DNA extraction are detected by the internal amplification control which is part of the RESEARCH Bacteria Mix. The amplification of the internal amplification control is detected at 610 nm (ROX™ channel).

4. Precautions

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 bacteria 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.

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 after rehydration or opening at < -18 ° C. Protect the RESEARCH Bacteria Mix from light.

Kit Component Label Information	Quantity 25 Reactions Order No. SMB95-1009	Quantity 100 Reactions Order No. SMB95-1010	Cap Color
RESEARCH Bacteria Mix	1 × lyophilized	4 × lyophilized	red
Rehydration Buffer	1 × 1.0 ml	4 × 1.0 ml	blue
Positive Control DNA	1 × lyophilized	1 × lyophilized	green
PCR grade Water	1 × 1.0 ml	1 × 1.0 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® RESEARCH Bacteria contains the reagents for the specific detection of bacteria. General industrial supplies and reagents, usually available in PCR laboratories are not included:

- qPCR device with filter sets for the detection of the fluorescence dyes FAM™ and ROX™ and suitable for 25 µl PCR reaction volumes
- DNA-free PCR reaction tubes for the specific qPCR device
- Microcentrifuge for 1.5 ml reaction tubes (i.e. Centrisart A-14C Microcentrifuge, Prod. No. A-14C-1EU)
- Pipettes with corresponding DNA-free filter tips to prepare and dispense the reaction mix (10, 100 and 1000 µl)
- Vortex mixer
- For DNA extraction, we recommend our Microsart® Bacteria Extraction kit, a unique DNA extraction method, which eliminates the risk of DNA contaminations, facilitating the detection of bacteria in cell culture and ATMPs via PCR (Prod. No. SMB95-2001)

7. Specimen

Microsart® RESEARCH Bacteria does not require DNA extraction prior use. Samples can be obtained directly from cell culture supernatants.

Samples directly received from cell cultures contain DNases which can degrade bacterial DNA even at lower temperatures. If the test cannot be performed immediately after sampling, samples should be stored at below -18 °C until use. Prior to performing the PCR, it is mandatory to stabilize the sample material by freezing or heat inactivation.

If you detect any inhibitory effects, DNA extraction i.e. with Microsart Bacteria Extraction (Prod No. SMB95-2001) is mandatory. If you work with native cell cultures that contain cells, please note that the sample for DNA extraction can contain up to 10^8 cells/ml. 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. For this, we recommend Microsart® Calibration Reagents (see Related Products for ordering information). All reagents and samples must be equilibrated to +2 to +8 °C prior use.

8.1 Rehydration of the reagents

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

1. RESEARCH Bacteria Mix Positive Control DNA	red cap green cap	Spin all lyophilized components for 5 sec at maximum speed of the microcentrifuge.
2. RESEARCH Bacteria Mix Positive Control DNA	red cap green cap	Add 600 µl Rehydration Buffer (blue cap). Add 300 µl PCR grade water (white cap).
3. RESEARCH Bacteria Mix Positive Control DNA	red cap green cap	Incubate 5 min at room temperature.

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 load.

1. Homogenize the rehydrated RESEARCH Bacteria Mix by vortexing. Spin briefly.
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

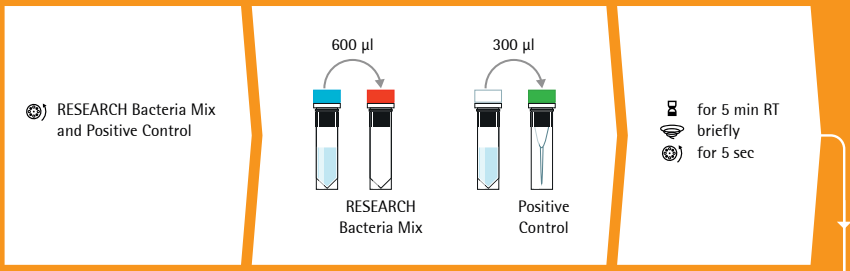
1. Load the cycler, check each PCR tube and the cycler lid for tight fit.
2. Program the qPCR cycler or check stored temperature profiles.
   See Appendix for temperature profiles of selected qPCR cyclers. Programs for additional cyclers might be available on request.
3. Start the program and data reading.

8.4 Analysis

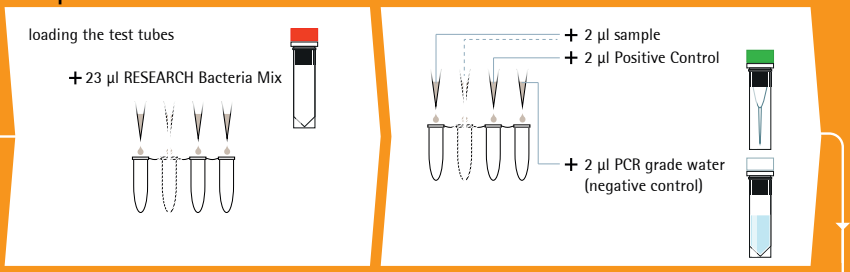
1. Save the data at the end of the run.
2. Show amplification plots for FAM™ and ROX™ in linear mode.
3. Set the threshold following the standard routine of the cycler or for selected cyclers as described in the Appendix. Check all fluorescence data for each sample for typical amplification curves (logarithmic increase of fluorescence) and correct threshold setting manually if needed.
4. Read the calculation of the Ct-values for the negative controls, the positive controls and the samples.

9. Short Instructions

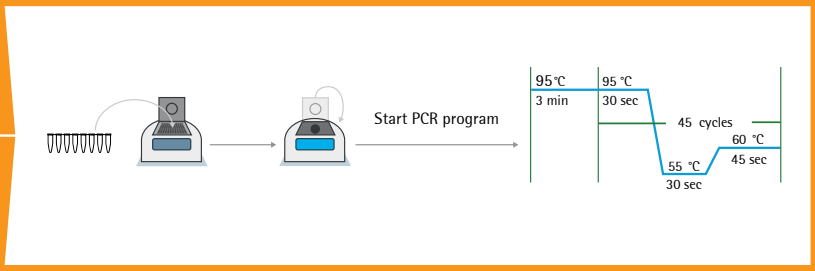
1. Rehydration of Reagents



2. Preparation of PCR Reactions



3. Starting the PCR Reaction



Rehydration Buffer
RESEARCH Bacteria Mix
PCR grade water
Positive Control

incubate
vortex
centrifuge
add

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

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

10. Notes on the Test Procedure

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

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

3. Inhibition may be caused by the sample matrix, but also by sample elution buffer of DNA extraction kits which are not recommended or validated. Negative controls should always be completed with the use of the sample elution buffer.

4. 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.

11. Interpretation of Results

The presence of bacterial DNA in the sample is indicated by an increasing fluorescence signal in the FAM™ channel during PCR. The concentration of the contaminant can be calculated by a software comparing the crossing cycle number of the sample with a standard curve created in the same run.
A successfully performed PCR without inhibition is indicated by an increasing fluorescence signal in the internal control channel. Bacterial 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 bacteria DNA loads in the sample.

Detection of Bacteria FAM™ channel	**Internal Control ROX™ channel**	**Interpretation**
positive ($C_t < 40$)	irrelevant	Bacteria positive
negative (no C_t)	negative (no C_t)	PCR inhibition
negative (no C_t)	positive ($C_t < 40$)	Bacteria negative
borderline ($C_t > 40$)	positive ($C_t < 40$)	result not valid, repeat process*
borderline ($C_t > 40$)	negative (no C_t)	PCR inhibition

* All samples showing a C_t value of > 40 need to be evaluated manually. Check the amplification curves for a significant increase of the fluorescence signal in comparison to the background noise of the Negative Control Sample. In case of amplification, the curve should form a typical logarithmic "amplification" curve. Adjust the threshold cycle manually. However, it is advised to repeat the testing of samples showing a borderline signal, as such a signal is not necessarily indicating the amplification of bacteria DNA but can be caused by sample matrix effects caused by incorrect sample preparations or setup errors.

12. Appendix

Assays of this kit can be performed with any type of real-time PCR cycler, able to detect the fluorescence dyes FAM™ and ROX™. The following qPCR cyclers were successfully tested with the Microsart® RESEARCH Bacteria kit:

Mx3005P®, CFX96 Touch, CFX96 Touch Deep Well, LightCycler® 480

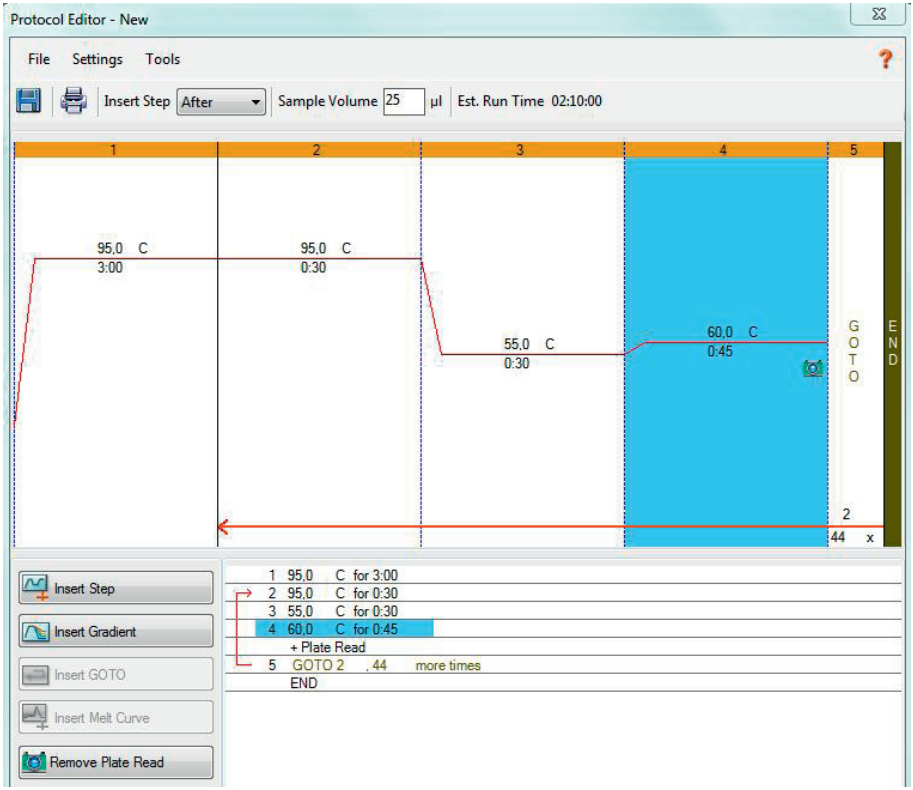
Programming and Data Recording of Different qPCR Devices

CFX96 Touch™ / CFX96 Touch™ deep well

Run Setup Protocol Tab:

- Click File --> New --> Protocol to open the Protocol Editor to 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

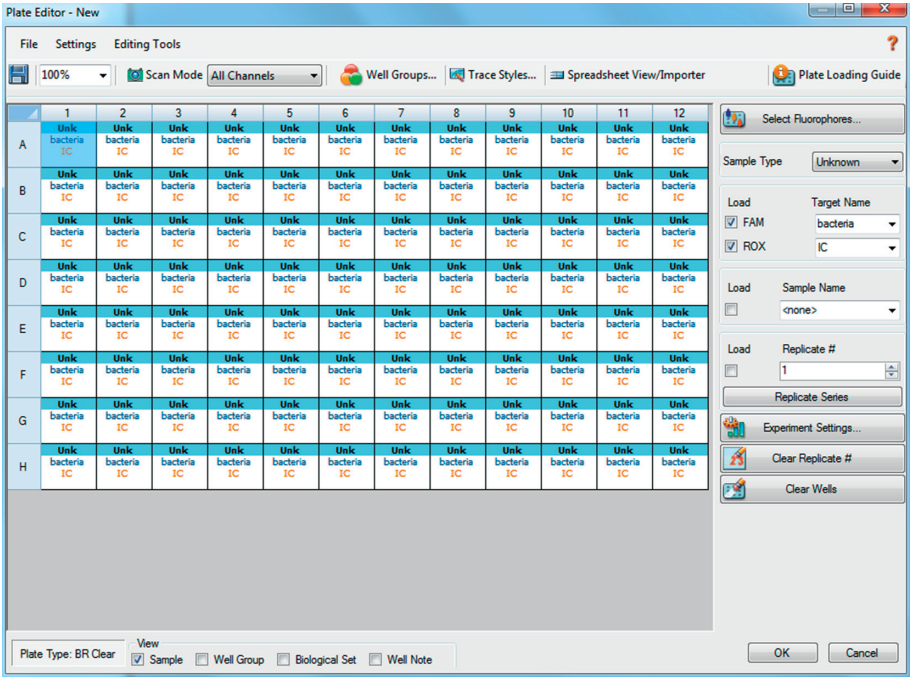
Segment 1:	1 cycle	3 min	95 °C	
Segment 2:		30 sec	95 °C	
Segment 3:		30 sec	55 °C	
Segment 4:		45 sec	60 °C	data collection
		GOTO Step 2	44 more cycles	



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 bacteria 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 bacteria 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
- View amplification curves of FAM™ channel by selecting the FAM™ checkbox under the amplification plot
- By right-clicking inside the amplification plot choose Baseline Threshold and set baseline cycles manually on basis of your positive control. Set Baseline Begin when fluorescence signal levelled off at a constant level. Set Baseline End before fluorescence signal of positive control increases
- Drag the threshold line manually to the initial linear section of the positive control
- Note specific Ct values

LightCycler® 480

Program 1: Pre-incubation	
Cycles	1
Analysis Mode	None
Temperature Targets	**Segment 1**
Target Temperature [°C]	95
Incubation time [s]	180
Temperature Transition Rate [°C/s]	4.4
Secondary Target Temperature [°C]	0
Step Size [°C]	0.0
Step Delay [Cycles]	0
Acquisition Mode	None

Program 2: Amplification			
Cycles	45		
Analysis Mode	Quantification		
Temperature Targets	**Segment 1**	**Segment 2**	**Segment 3**
Target Temperature [°C]	95	55	60
Incubation time [s]	30	30	45
Temperature Transition Rate [°C/s]	4.4	2.2	4.4
Secondary Target Temperature [°C]	0	0	0
Step Size [°C]	0	0	0
Step Delay [Cycles]	0	0	0
Acquisition Mode	None	None	Single

Program 3: Cooling	
Cycles	1
Analysis Mode	None
Temperature Targets	**Segment 1**
Target Temperature [°C]	40
Incubation time [s]	30
Temperature Transition Rate [°C/s]	2.2
Secondary Target Temperature [°C]	0
Step Size [°C]	0
Step Delay [Cycles]	0
Acquisition Mode	None

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: 45 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"

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 registrated trademark of a member of the Roche Group. ABI Prism is a registrated trademark of Applera Corporation or its subsidiaries in the US and certain other countries. FAM™ and ROX™ are trademarks of Applera Corporation or it's subsidiaries in the US and certain other countries. Mx3005P is a trademark of Agilent Technologies. CFX96 Touch is a trademark of Bio Rad Laboratories, Inc. Microsart is a registered trademark of Sartorius Stedim Biotech. Myocoplasma Off and PCR Clean are trademarks of Minerva Biolabs GmbH, Germany.

Last technical revision: 2017-06-21

13. 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
SMB95-1008	Microsart® ATMP Bacteria	100 tests

Microsart® Calibration Reagent, 1 vial, 10^8 genomes / vial

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	Micrococcus luteus / Kocuria rhizophila
SMB95-2033	Clostridium sporogenes
SMB95-2034	Bacteroides vulgatus
SMB95-2035	Staphylococcus aureus

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

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
SMB95-2005	Bacillus subtilis
SMB95-2006	Pseudomonas aeruginosa
SMB95-2007	Micrococcus luteus / Kocuria rhizophila
SMB95-2008	Clostridium sporogenes
SMB95-2009	Bacteroides vulgatus
SMB95-2010	Staphylococcus aureus

DNA Extraction Kits

SMB95-2001	Microsart® Bacteria Extraction	50 extractions
SMB95-2003	Microsart® AMP Extraction (only for Mycoplasma qPCR)	50 extractions

PCR Clean™ (formerly DNA Remover™) *

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

Mycoplasma Off™ *

15-1000	Surface Disinfectant Spray, spray bottle	1000 ml
15-5000	Surface Disinfectant Spray, refill bottles	5x 1000 ml

Mycoplasma Off™ Wipes*

15-1001	Surface Disinfectant Wipes	120 wipes
15-5001	Surface Disinfectant Wipes, refill sachets	5 x 120 wipes

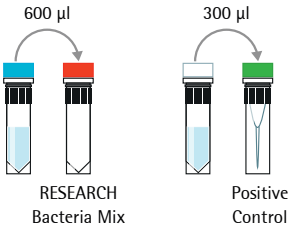
*** Distributed by Minerva Biolabs**

Procedure-Overview

Microsart® RESEARCH Bacteria

1. Rehydration of Reagents

RESEARCH Bacteria Mix and Positive Control

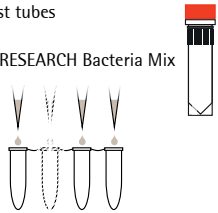


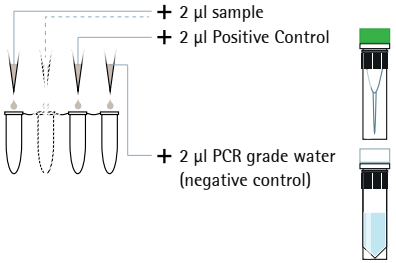
for 5 min RT
briefly
for 5 sec

2. Preparation of PCR Reactions

loading the test tubes

+ 23 µl RESEARCH Bacteria Mix





3. Starting the PCR Reaction

Rehydration Buffer
RESEARCH Bacteria Mix
PCR grade water
Positive Control

incubate
vortex
centrifuge
add

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

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

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