Instructions for Use

Microsart® Bacteria Extraction

Bacterial DNA extraction kit

Prod. No. SMB95-2001

Reagents for 50 extractions

For use in research and quality control

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 50 tests
	Manufacturer

Contents

1. Intended Use

Microsart® Bacteria Extraction kit introduces a state-of-the-art DNA extraction method for DNA extraction from cell cultures and cell culture derived biologicals, like autologous transplants and other advanced therapy medicinal products (ATMP), for subsequent DNA amplification via PCR.

2. Explanation of the Test

To achieve highest sensitivity and to avoid inhibitory effects in PCR testing, a DNA extraction is recommended. For most test materials, DNA extraction methods are available providing templates suitable for PCR. However, most of the DNA extraction kits available on the market are not free of bacterial DNA contaminations. Microsart® Bacteria Extraction introduces a unique DNA extraction method, which eliminates the risk of DNA contaminations, facilitating the detection of bacteria in cell culture and ATMPs via PCR.

The extraction procedure can be performed within 1 hour. In contrast to the culture method, samples do not need to contain vital bacteria, as all intact bacteria (e.g. live, dormant, non-culturable etc.) are detected.

3. Test Principle

Microsart® Bacteria Extraction kit was optimized for the extraction of genomic bacterial DNA from different sample matrices including cell culture samples. The contamination risk has been reduced to a minimum due to less handling steps.

An internal amplification control DNA from the Microsart® ATMP Bacteria kit can be added to the sample prior DNA extraction to monitor the extraction process by detecting false negative results which can occur due to improper DNA extraction or PCR inhibition. Alternatively, the internal control DNA can be added directly to the Mastermix during PCR setup.

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 bacteria strains, the local regulatory requirements for S2 labs must be considered.

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

6. This extraction kit has been validated with 1 ml starting volume. If you use less than 1 ml it must be ensured that 99 cfu can be detected in the appropriate volume.

7. This extraction kit is not suitable for the extraction of mycoplasma DNA. Therefore the DNA extract of this kit cannot be used for mycoplasma qPCR analysis.

8. This leaflet must be widely understood for a successful use of Microsart® Bacteria Extraction kit. 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. For each test setup, at least one negative extraction control should be included. Positive controls facilitate the evaluation of the test.

11. The controls should be carried out in the same manner as the samples.

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 cleaned thoroughly with PCR Clean™ (Minerva Biolabs, Prod. No. 15-2025) or PCR Clean™ Wipes (Minerva Biolabs, Prod. No. 15-2001) before and during the working process.

3. All materials which are introduced into the isolator/glovebox should be cleaned thoroughly with PCR Clean™. Don´t forget to clean the airlock with PCR Clean™. Pipettes and gloves should be cleaned thoroughly with PCR Clean™ Wipes prior and during the process.

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

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

5. Reagents

Each kit contains reagents for 50 extractions. The expiry date of the unopened package is marked on the package label. The kit components are stored at ambient temperature until use. Suspension Buffer should be stored at 2 - 8 °C after first use.

Kit Component Label Information	50 Extractions Order No. SMB95-2001	Cap Color
Lysis Buffer	2 x 13 ml	transparent
Suspension Buffer	4 x 1.5 ml	violet
Processing Tubes	50 Tubes	

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

6. Needed but not Included

Microsart® Bacteria Extraction kit contains reagents for sample collection and DNA extraction. 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 DNA (we recommend Biosphere® filter tips from Sarstedt: 0.5-20 µl, 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
- Microcentrifuge for 1.5 ml reaction tubes (Centrisart A-14, Prod. No. A-14-1EU)
- Vortex Mixer
- Pipettes
  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

For subsequent PCR analysis, the following consumables and equipment are required additionally:

- Bacterial DNA PCR detection system. We recommend the Microsart® ATMP Bacteria kit (Sartorius Prod. No. SMB95-1008), or the Microsart® RESEARCH Bacteria kit (Sartorius Prod. No. SMB95-1009/1010), both qPCR-based methods, designed for the direct detection of bacteria in cell cultures and cell culture derived biologicals

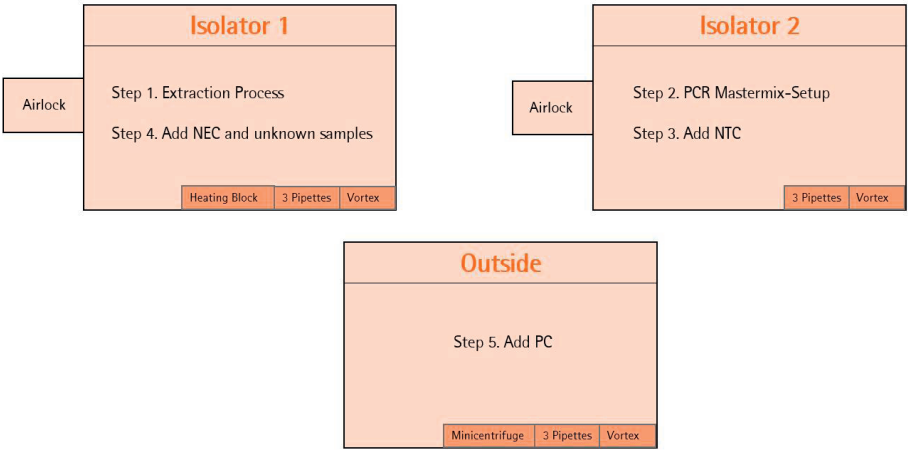
- qPCR device with filter sets for the detection of the fluorescence dyes FAM™ and ROX™ and suitable for 25 µl PCR reaction volumes

- DNA-free PCR reaction tubes that must be free from bacterial DNA for the specific qPCR device (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)

- Minicentrifuge for PCR-tubes

- Isolator/glovebox (for PCR-setup)
- Vortex mixer
- Set of 3 pipettes
- Rack for 1.5 ml tubes
- Rack for PCR tubes

Schematical Overview of technical setup and experimental design

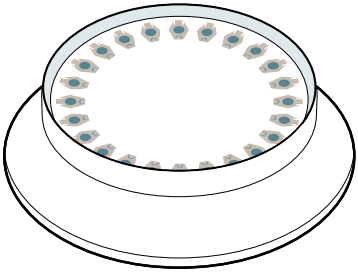


It is also possible to connect Isolator 1 and Isolator 2 via an airlock so that you can transfer your PCR-tubes after Step 3 directly from Isolator 2 into Isolator 1. Please note that you need an additional airlock for Isolator 2 in this case.

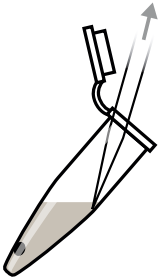
7. Specimen

Sample Collection and Storage

1. max. 1 ml liquid of cell culture or cell culture supernatant material is transferred into a provided DNA-free 1.5 ml processing tube (transparent cap).
   Attention: we recommend a maximum cell content of 10^6 cells/ml.
2. Spin down supernatant for 15 minutes at a speed of at least 16,200 x g to sediment bacteria particles.
   Attention: Make sure to position the tubes in the centrifuge in order to form the pellet on the back side of the tube, as explained on the figure below.
3. Discard the supernatant carefully and completely as explained on the figure below. Proceed to DNA Extraction. If DNA extraction cannot be performed immediately, freeze samples at ≤ -18 °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 form the pellet on the back wall of the tube.



Slowly discard all the supernatant without disturbing the pellet.

8. Test Procedure

8.1 Recommendation for product release testing

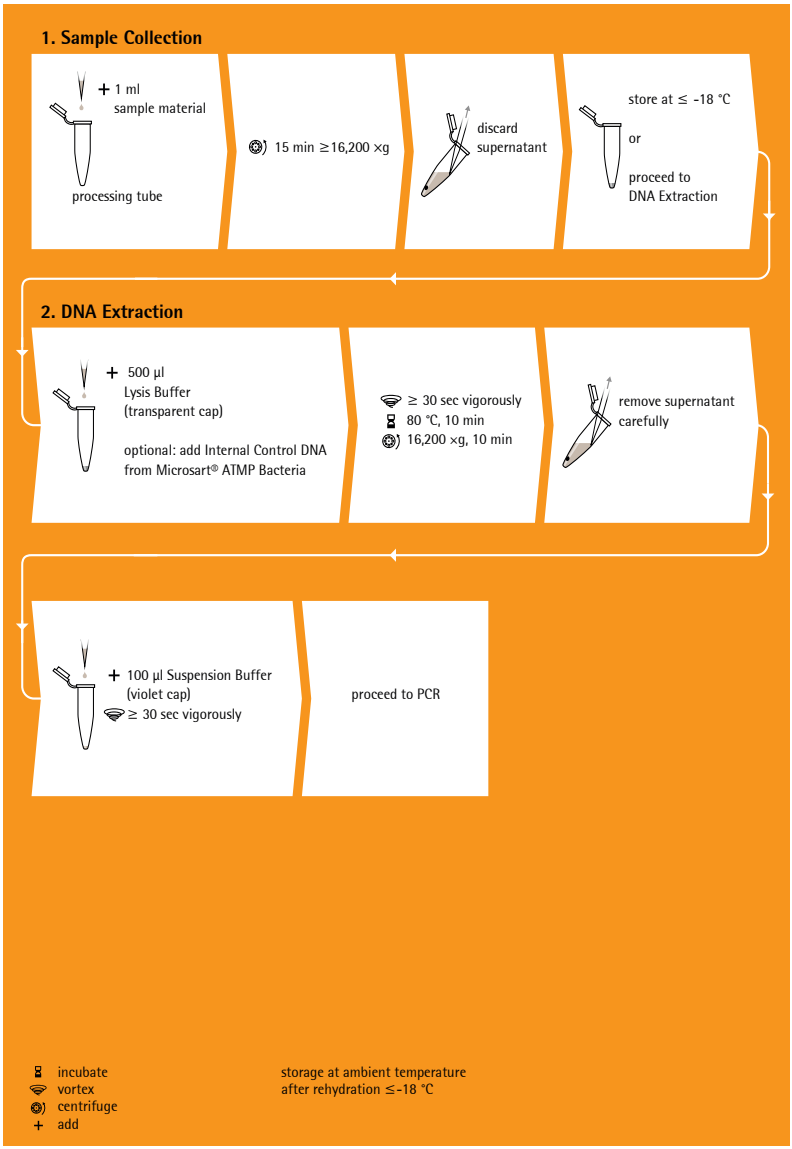
The extraction process should be carried out with a negative extraction control (NEC) and samples in duplicates (= 3 extractions for 1 product).

8.2 DNA Extraction Process

1.	Add 500 µl Lysis Buffer (transparent cap) to cell pellet. Recommended for users of Microsart® ATMP Bacteria detection kit: The Internal Control DNA which is included in the Microsart® ATMP Bacteria detection kit can also be used to monitor the extraction process. Add 20 µl 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 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 on the figure Chapter 7.
5.	Remove supernatant carefully and completely following the explanations in paragraph 7. Make sure to not withdraw the pellet in the process. Attention: There is a high risk of inhibition in PCR analysis if residues remain in the tube.
6.	Add 100 µl 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 ≤ -18 °C. Repeated freezing and thawing should be avoided.

9. Short Instructions



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
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11. 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
SMB95-1007	Microsart® ATMP Bacteria (patient)	10 patients

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
SMB95-2033	Clostridium sporogenes
SMB95-2034	Bacteroides vulgatus
SMB95-2035	Staphylococcus aureus
SMB95-2036	Mycoplasma salivarium

Microsart® Validation Standard, 3 vials each, 10 CFU/vial for Mollicutes (SMB95-2011 - SMB95-2020) and 99 CFU/vial for other bacterial species (SMB95-2005 - SMB95-2010)

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	
SMB95-2008	Clostridium sporogenes	
SMB95-2009	Bacteroides vulgatus	
SMB95-2010	Staphylococcus aureus	
SMB95-2020	Mycoplasma salivarium	
DNA Extraction Kit		
SMB95-2003	Microsart® AMP Extraction (only for Mycoplasma qPCR)	50 extractions
PCR Clean™ *		
15-2025	DNA Decontamination Reagent, spray bottle	250 ml
15-2200	DNA Decontamination Reagent, refill bottles	4x 500 ml
PCR Clean™ Wipes*		
15-2001	DNA Decontamination Wipes	120 wipes
15-2002	DNA Decontamination Wipes, refill sachets	5 x 120
Mycoplasma Off™ *		
15-1000	Surface Disinfectant Spray, spray bottle	1000 ml
15-5000	Surface Disinfectant Spray, refill bottles	5000 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® Bacteria Extraction

1. Sample Collection

+ 1 ml
sample material

processing tube

15 min ≥16,200 ×g

discard
supernatant

store at ≤ -18 °C

or

proceed to
DNA Extraction

2. DNA Extraction

+ 500 µl
Lysis Buffer
(transparent cap)

optional: add Internal Control DNA
from Microsart® ATMP Bacteria

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

remove supernatant
carefully

+ 100 µl Suspension Buffer
(violet cap)
≥ 30 sec vigorously

proceed to PCR

incubate
vortex
centrifuge
+ add

storage at ambient temperature
after rehydration ≤-18 °C

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

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