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.

Expiration date

Store at

Contains reagents for 50 tests

Manufacturer
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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.

<table>
<thead>
<tr>
<th>Kit Component</th>
<th>50 Extractions</th>
<th>Cap Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysis Buffer</td>
<td>2 x 13 ml</td>
<td>transparent</td>
</tr>
<tr>
<td>Suspension Buffer</td>
<td>4 x 1.5 ml</td>
<td>violet</td>
</tr>
<tr>
<td>Processing Tubes</td>
<td>50 Tubes</td>
<td></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® 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 (Centrifuge 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.
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 $\leq -18^\circ$C. Repeated freezing and thawing should be avoided.
   Attention: Samples can only be inactivated or frozen after this sample collection step.

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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

1. Sample Collection

+ 1 ml sample material

processing tube

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 x g, 10 min

≥ 30 sec vigorously

proceed to PCR

+ 100 µl Suspension Buffer (violet cap)

proceed to PCR

incubate

vortex

centrifuge

add

storage at ambient temperature after rehydration ≤ -18 °C
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
Microsart is a registered trademark of Sartorius Stedim Biotech. Mycoplasma Off and PCR Clean™ are trademarks of Minerva Biolabs GmbH, Germany.

Last technical revision: 2018-04-19
11. Related Products

**Detection Kits for qPCR**

<table>
<thead>
<tr>
<th>SKU</th>
<th>Description</th>
<th>Tests/Count</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>SMB95-1009</td>
<td>Microsart® RESEARCH Bacteria</td>
<td>25 tests</td>
</tr>
<tr>
<td>SMB95-1008</td>
<td>Microsart® ATMP Bacteria</td>
<td>100 tests</td>
</tr>
<tr>
<td>SMB95-1007</td>
<td>Microsart® ATMP Bacteria (patient)</td>
<td>10 patients</td>
</tr>
</tbody>
</table>

**Microsart® Calibration Reagent**, 1 vial, $10^9$ genomes / vial

<table>
<thead>
<tr>
<th>SKU</th>
<th>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>
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<tr>
<td>SMB95-2024</td>
<td>Mycoplasma pneumoniae</td>
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<tr>
<td>SMB95-2025</td>
<td>Mycoplasma synoviae</td>
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<td>SMB95-2026</td>
<td>Mycoplasma fermentans</td>
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<td>SMB95-2027</td>
<td>Mycoplasma hyorhinis</td>
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<td>SMB95-2028</td>
<td>Acholeplasma laidlawii</td>
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<td>SMB95-2029</td>
<td>Spiroplasma citri</td>
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<td>SMB95-2030</td>
<td>Bacillus subtilis</td>
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<td>SMB95-2031</td>
<td>Pseudomonas aeruginosa</td>
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<td>SMB95-2032</td>
<td>Micrococcus luteus</td>
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<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>
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</tbody>
</table>

**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)

<table>
<thead>
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<th>SKU</th>
<th>Description</th>
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<tr>
<td>SMB95-2011</td>
<td>Mycoplasma arginini</td>
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<td>SMB95-2012</td>
<td>Mycoplasma orale</td>
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<td>SMB95-2013</td>
<td>Mycoplasma gallisepticum</td>
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<td>SMB95-2014</td>
<td>Mycoplasma pneumoniae</td>
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<tr>
<td>SMB95-2015</td>
<td>Mycoplasma synoviae</td>
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<tr>
<td>SMB95-2016</td>
<td>Mycoplasma fermentans</td>
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<tr>
<td>SMB95-2017</td>
<td>Mycoplasma hyorhinis</td>
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<tr>
<td>SMB95-2018</td>
<td>Acholeplasma laidlawii</td>
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<tr>
<td>SMB95-2019</td>
<td>Spiroplasma citri</td>
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<tr>
<td>SMB95-2020</td>
<td>Bacillus subtilis</td>
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<tr>
<td>SMB95-2021</td>
<td>Pseudomonas aeruginosa</td>
</tr>
</tbody>
</table>
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  50 extractions
(only for Mycoplasma qPCR)

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
1. Sample Collection

- + 1 ml sample material
- processing tube
- 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 xg, 10 min
- remove supernatant carefully

- + 100 µl Suspension Buffer (violet cap)
- ≥ 30 sec vigorously
- proceed to PCR

storage at ambient temperature after rehydration ≤ -18 °C

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