Microsart® AMP Extraction

Genomic DNA Extraction Kit
Version 2
Prod. No. SMB95-2003

Reagents for 50 extractions
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

The Microsart® AMP Extraction kit is intended for the isolation of mycoplasma DNA from different types of sample material, particularly cell culture supernatants. The kit was specifically designed to complement the Microsart® AMP Mycoplasma, Microsart® ATMP Mycoplasma and Microsart® RESEARCH Mycoplasma qPCR kits and to provide best performance in terms of sensitivity and robustness for the detection of mycoplasma in a test sample.

2. Test Principle

The method is simple and consists of four general steps:

1. Cell lysis
2. Selective binding of DNA to spin columns
3. Removal of residual contaminants and inhibitors
4. Elution of purified DNA

The procedure does not require phenol/chloroform extraction and needs minimal hands-on time. The purified DNA is ready-to-use for PCR.

3. Notes on the Test Procedure

1. This leaflet must be fully understood in order to successfully use Microsart® AMP 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 must not be used beyond their shelf life.

2. Any deviation from the extraction method may affect the results.

3. The use of control samples is advised to secure the day-to-day validity of results. Sample spiking with an Internal Amplification Control facilitates the evaluation of the extraction performance.

4. Do not use other alcohols apart from ethanol as it will lead to inconsistent results.

5. Pre-heating the Buffer D to 70 °C will increase the DNA elution yield.

6. Untreated cell culture materials should be extracted as soon as possible. After stabilization by heat treatment (95 °C, 10 min, up to 500 μl), cell culture materials can be stored for 1 week at room temperature.
### 4. Reagents

Each kit contains components and reagents for 50 extractions. The expiry date of the kit is given on the box label. The kit components must be stored at room temperature (18 to 25 °C).

<table>
<thead>
<tr>
<th>Kit Component Label Information</th>
<th>Quantity</th>
<th>Required Supplements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spin Columns</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Collection Tubes</td>
<td>3 × 50</td>
<td></td>
</tr>
<tr>
<td>Sample Storage Tubes</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Buffer A1</td>
<td>15 ml</td>
<td></td>
</tr>
<tr>
<td>Buffer A2</td>
<td>25 ml</td>
<td></td>
</tr>
<tr>
<td>Buffer B (concentrate)</td>
<td>15 ml</td>
<td>15 ml ethanol</td>
</tr>
<tr>
<td>Buffer C (concentrate)</td>
<td>12 ml</td>
<td>48 ml ethanol</td>
</tr>
<tr>
<td>Buffer D</td>
<td>2 × 2 ml</td>
<td></td>
</tr>
</tbody>
</table>

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

Microsart® AMP Extraction contains all the reagents and components for the extraction of mycoplasma DNA from various sample materials. Additional consumables and equipment are supplied by the user:

**Consumables**
- Ethanol > 96 % abs.
- 1.5 ml reaction tubes, DNA- and RNA-free

**Equipment**
- Microcentrifuge and heat block with shaking function for 1.5 ml reaction tubes
- Vortex
- 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:
- Proteinase K (see chapter 7.1 and “Related Products”)
- Microsart® AMP Coating Buffer (see chapter 7.1 and “Related Products”)

Needed but not included 7
6. Precautions

- The Microsart® AMP Extraction Kit is intended for use in research and quality control only— not for clinical use or diagnostics.

- This kit should be used by trained laboratory staff only.

- All samples should be considered potentially infectious and handled with all due care and attention.

- The kit substances may be disposed of according to local regulations.

- Always wear a suitable lab coat, disposable gloves, and protective goggles.

- The sample preparation waste contains Buffer A2 and Buffer B, which can form highly reactive compounds when combined with bleach. DO NOT add bleach or acidic solutions directly to the sample preparation waste. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water.

The hazard (H) statements for Buffer A2 are:
H225 Highly flammable liquid and vapour.
H315 Causes skin irritation.
H318 Causes serious eye damage.
H336 May cause drowsiness or dizziness.
H411 Toxic to aquatic life with long lasting effects.

The hazard (H) statements for Buffer B are:
H302 Harmful if swallowed.
H314 Causes severe skin burns and eye damage.
H412 Harmful to aquatic life with long lasting effects.
7. Test Procedure

Before first use, reconstitute Buffer B and Buffer C with absolute ethanol as indicated on the bottle label. Pre-heat Buffer D to 70 °C to increase the yield of DNA.

Importantly, this extraction method has been validated in combination with the qPCR kits Microsart® ATMP Mycoplasma and Microsart® AMP Mycoplasma. As starting material, a collection of several representative cell culture samples (e.g. supernatants, suspensions etc.) has been provided by our customers showing that the assay can be successfully performed with different types of cell culture-derived material. Therefore, the maximum cell number can vary significantly according to the specific characteristics of the sample (e.g. medium, cell type, and a combination of both factors) and suitability should be checked as part of each matrix specific validation.

7.1 Preparation of Cell Culture Material

The extraction process should be carried out with a negative extraction control (NEC) and samples in duplicates.

1. Transfer 200 μl of cell culture material into a new 1.5 ml reaction tube.

   **Attention:** For low-complexity matrices as aqueous samples (e.g. H₂O, Tris Buffer), add 80 μl of Microsart® AMP Coating Buffer to the sample and briefly vortex. Then, process the whole volume (280 μl). This implies loading the sample onto the spin column (see below: chapter 7.2.1, DNA Isolation) in 2 steps to avoid overloading and spilling (column max. volume 850 μl).

   **Optional process control:** The Internal Control DNA of the qPCR kit can be added here to assess the extraction performance. If Microsart® ATMP Mycoplasma Kit or Microsart® AMP Mycoplasma Kit will be used in combination with this extraction kit:
   - Microsart® ATMP Mycoplasma Kit: add 12 μl of the supplied Internal Control DNA to each 200 μl sample.
   - OR
   - Microsart® AMP Mycoplasma Kit: add 5 μl of the supplied Internal Control DNA to each 200 μl sample.

   Then process the whole volume (212 μl OR 205 μl) and proceed to step 2.

2. Add 200 μl of Buffer A1, vortex briefly.

   For samples with high protein content (concentration >10 mg/ml), an additional proteinase treatment is needed. Proceed as follows:
   - Add 10 μl of Proteinase K (see “Related Products” for ordering information).
3. Incubate at 70 °C for 10 min. For this step, we recommend using a heat block with continuous shaking of the sample. Alternatively, vortex the sample 3 to 4 times at regular intervals during the incubation. Equilibrate at room temperature for 2 min. Then spin down briefly to remove drops from the inside of the lid.

4. Add 400 μl of Buffer A2 to the mixture. Vortex immediately and thoroughly.

5. Proceed immediately as described in the chapter “DNA Isolation”.

7.2 DNA Isolation

1. Place a spin column into a collection tube and label the spin column lid with the sample ID. Transfer the sample lysate-mixture into the spin column without wetting the rim.

2. Centrifuge for 1 min at ≥ 10,000 × g. Discard the flow-through from the collection tube and reassemble the spin column and the collection tube.

3. Add 500 μl of Buffer B. Centrifuge for 1 min at ≥ 10,000 × g. Discard the collection tube and place the spin column into a new collection tube.

4. Add 500 μl of Buffer C. Centrifuge for 1 min at ≥ 10,000 × g. Discard the collection tube and place the spin column into a new collection tube.
   **Optional:** Repeat the wash step with Buffer C.

5. Centrifuge for 3 min at max. speed to remove any remaining Buffer C.

6. Discard the collection tube and place the spin column into a sample storage tube.

7. Pipet 60 μl of the pre-heated Buffer D directly at the center of the silica membrane of the spin column. This volume of Buffer D is enough to cover the surface of the membrane entirely. Incubate for 2 min at room temperature.

8. Following the incubation, centrifuge for 2 min at 8,000 × g.

9. Discard the spin column and use the eluate directly for PCR (recommended). However, if samples cannot be processed immediately after extraction, the eluted DNA can be stored for 6 days at +2 °C to +8 °C. If long term storage is required, store at ≤ -18 °C. Repeated freezing and thawing should be avoided.
8. Related Products

Detection Kits for qPCR

<table>
<thead>
<tr>
<th>Code</th>
<th>Product Description</th>
<th>Tests/Samples</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>
<tr>
<td>SMB95-1014/1013</td>
<td>Microsart® RESEARCH Fungi</td>
<td>25/100 tests</td>
</tr>
</tbody>
</table>

Microsart® Calibration Reagent, $10^8$ genomes / vial, 1 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>
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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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<tr>
<td>SMB95-2026</td>
<td>Mycoplasma fermentans</td>
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<tr>
<td>SMB95-2027</td>
<td>Mycoplasma hyorhinis</td>
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<tr>
<td>SMB95-2028</td>
<td>Acholeplasma laidlawii</td>
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<tr>
<td>SMB95-2029</td>
<td>Spiroplasma citri</td>
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<tr>
<td>SMB95-2030</td>
<td>Bacillus subtilis</td>
</tr>
<tr>
<td>SMB95-2031</td>
<td>Pseudomonas aeruginosa</td>
</tr>
<tr>
<td>SMB95-2032</td>
<td>Kocuria rhizophila</td>
</tr>
<tr>
<td>SMB95-2033</td>
<td>Clostridium sporogenes</td>
</tr>
<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, $10^6$ genomes / vial, 1 vial (fungi)

<table>
<thead>
<tr>
<th>Code</th>
<th>Product Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMB95-2044</td>
<td>Candida albicans</td>
</tr>
<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>
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<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, 10 CFU / vial, 3 vials each (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>
<tr>
<td>SMB95-2015</td>
<td>Mycoplasma synoviae</td>
</tr>
<tr>
<td>SMB95-2016</td>
<td>Mycoplasma fermentans</td>
</tr>
<tr>
<td>SMB95-2017</td>
<td>Mycoplasma hyorhinis</td>
</tr>
</tbody>
</table>
SMB95-2018  Acholeplasma laidlawii
SMB95-2019  Spiroplasma citri
SMB95-2020  Mycoplasma salivarium

**Microsart® Validation Standard, 99 CFU / vial, 6 vials each (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
SMB95-2002  Microsart® AMP Coating Buffer  20 × 2 ml
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 × 500 ml

**PCR Clean™ Wipes**

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

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Trademarks
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Last technical revision: 2016-08-17
1. Preparation of Cell Culture Material

- Add 200 μl sample
- Add 200 μl Buffer A1
- Incubate for 10 min at 70 °C
- Vortex & Centrifuge for 10 sec
- Add 400 μl Buffer A2
- Incubate

2. DNA Isolation

- Transfer sample
- Centrifuge at 10,000 × g for 1 min
- Discard flow-through
- Add 500 μl Buffer B
- Centrifuge at 10,000 × g for 1 min
- Change tube
- Add 500 μl Buffer C
- Centrifuge at 10,000 × g for 1 min
- Change tube
- Add 60 μl Buffer D
- Incubate for 2 min at RT
- Centrifuge at 8,000 × g for 2 min
- Change tube

storage +18 - +25 °C (RT)

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

ST_SI_Microart®-AMP-Extraction_02_EN

Short Instructions