SARTORIUS

Instructions for Use

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:

MB minerva biolabs®

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

Contents

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).

Kit Component Label Information	Quantity	Required Supplements
	50 Extractions Order No. SMB95-2003	
Spin Columns	50	–
Collection Tubes	3 × 50	-
Sample Storage Tubes	50	-
Buffer A1	15 ml	-
Buffer A2	25 ml	-
Buffer B (concentrate)	15 ml	15 ml ethanol
Buffer C (concentrate)	12 ml	48 ml ethanol
Buffer D	2 × 2 ml	-

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")

6. Precautions

- The Microsart® AMP Extraction Kit is intended for 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. H_2O, 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

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
SMB95-1007	Microsart® ATMP Sterile Release	10 samples
SMB95-1008	Microsart® ATMP Bacteria	100 tests
SMB95-1009	Microsart® RESEARCH Bacteria	25 tests
SMB95-1012	Microsart® ATMP Fungi	100 tests
SMB95-1014/1013	Microsart® RESEARCH Fungi	25/100 tests

Microsart® Calibration Reagent, 10^8 genomes / vial, 1 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, 10^6 genomes / vial, 1 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, 10 CFU / vial, 3 vials each (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, 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

** Distributed by Minerva Biolabs

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1. Preparation of Cell Culture Material

+ 200 µl sample
+ 200 µl Buffer A1
for 10 sec

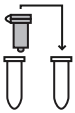
& for 10 min at 70 °C

+ 400 µl Buffer A2
for 10 sec

2. DNA Isolation

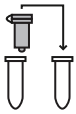
Transfer sample
10,000 × g for 1 min
Discard flow-through

+ 500 µl Buffer B
10,000 × g for 1 min



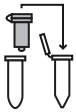
Change tube

+ 500 µl Buffer C
10,000 × g for 1 min



Change tube

max. speed for 3 min



Change tube

+ 60 µl Buffer D
for 2 min at RT
8,000 × g for 2 min

DNA ready for PCR

incubate
vortex
centrifuge
add
shake

storage +18 - +25 °C (RT)

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