SARTORIUS

Instructions for Use

Microsart® ATMP Extraction

Bacterial, fungi, and yeast DNA extraction kit
Prod. No. SMB95-2001

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 50 extractions
	Manufacturer

Contents

1. Intended Use

Microsart® ATMP Extraction kit introduces a state-of-the-art DNA extraction method for the isolation of bacterial, fungal, yeast DNA from cell cultures and cell culture-derived biologicals, like autologous transplants and other advanced therapy medicinal products (ATMP). The kit allows obtaining DNA templates for subsequent DNA amplification via PCR (e.g. for contamination detection, product release testing etc.).

2. Explanation of the Test

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

The extraction procedure can be performed within 1 hour. In contrast to culture-based methods, for PCR tests, samples do not need to contain living material, as all intact particles (e.g. live, dormant, non-culturable etc.) are detected.

3. Test Principle

Microsart® ATMP Extraction kit was optimized for the extraction of genomic bacterial or fungal DNA from different sample matrices, including cell culture samples. The contamination risk has been minimized thanks to a reduced number of handling steps and a straightforward protocol.

An internal control DNA, provided with the Microsart® ATMP Bacteria or Microsart® ATMP Fungi kit, can be added to each sample prior to DNA extraction to monitor the extraction process. This will allow detection of false negative results, which may occur due to improper DNA extraction or PCR inhibition. Alternatively, the internal control DNA can be added directly to the master mix 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 by trained staff, only. A clean lab coat and disposable gloves should be worn 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 work with living strains, the local regulation for S2 labs must be followed.

5. Aliquoting and repeatedly freezing and thawing the samples can increase the risk of sample contaminations. Therefore, this should be avoided whenever 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 still the required sensitivity is reached with the reduced starting volume.

7. This kit is not validated for the extraction of mycoplasma DNA.

8. This leaflet must be widely understood for a successful use of Microsart® ATMP 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 handled in the same manner as the samples.

12. Participation in external quality control programs, such as those offered by Minerva Biolabs GmbH (www.minerva-biolabs.com), is recommended.

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 and all materials introduced into the isolator/glovebox should be thoroughly decontaminated with a chlorine-based cleaning agent, e.g. Contec® ProChlor, before and during the work process. Do not forget to clean the airlock. Pipettes and gloves should also be cleaned thoroughly before and during the process.

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

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

5. Always use a new, unopened DNA-free pipette filter tip-box for each assay. Reaction vials should be closed immediately after every pipetting step.

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.
The lot specific Certifices of Analysis can be downloaded from the MySartorius portal (https://my.sartorius.com).

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

6. Needed but not included

Microsart® ATMP 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
- Chlorine-based cleaning agents, e.g. Contec® ProChlor
- DNA-free pipette filter tips (we recommend Biosphere® filter tips from Sarstedt: 0.5-20 µl, Prod. 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 with optional shaking function
- Microcentrifuge 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
- Rack for 1.5 ml tubes

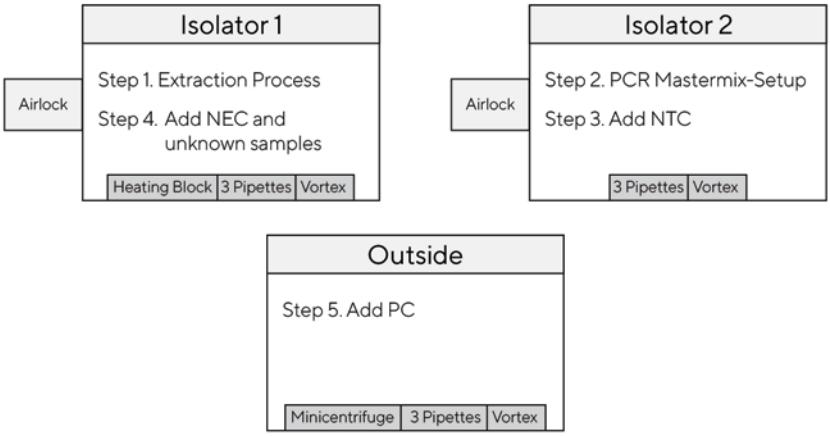
For subsequent PCR analysis, the following consumables and equipment are additionally required:

- PCR-based system for the detection of bacterial DNA: we recommend the Microsart® ATMP Bacteria kit (Sartorius Prod. No. SMB95-1008), or the Microsart® Research Bacteria kit (Sartorius Prod. No. SMB95-1009).
- PCR-based system for the detection of fungi and yeasts DNA: we recommend the Microsart® ATMP Fungi kit (Sartorius Prod. No. SMB95-1012), or the Microsart® Research Fungi kit (Sartorius Prod. No. SMB95-1013/1014).

Note: All these kits are qPCR-based assays, designed and optimized for the detection in cell cultures and cell culture-derived biologicals.

- qPCR device with filter sets for the detection of the fluorescent dyes FAM™ and ROX™ and suitable for 25 µl PCR reaction volumes.
- Minicentrifuge for PCR tubes
- Isolator/glovebox (for PCR setup)
- Vortex
- Set of 3 pipettes (see previous page)
- Rack for 1.5 ml tubes and for PCR tubes

Schematic overview of technical setup and experimental design:



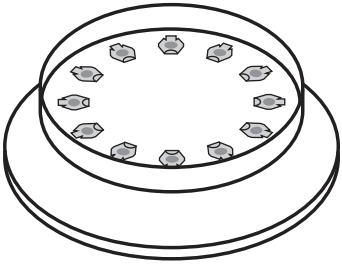
It is also possible to connect Isolator 1 and Isolator 2 via an airlock so that you can transfer the PCR tubes after Step 3 directly from Isolator 2 into Isolator 1. Please note that in this case you would need an additional airlock for Isolator 2.

7. Specimen

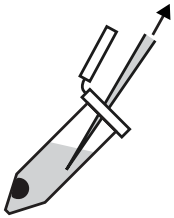
Sample collection and storage

The kit has been validated using a maximum cell concentration of 10^6 cells/ml. Notably, the assay can be performed with different types of cell culture-derived material. Therefore, the optimal sampling parameters, like volume or cell number, can vary according to the specific characteristics of the sample (e.g. medium, cell type) and may require optimization of the procedure.

1. max. 1 ml of cell culture or cell culture supernatant liquid material is transferred into a provided DNA-free 1.5 ml processing tube.
2. Spin down for 15 min at a speed of at least 16,200 × g to sediment particles.
   Attention: Make sure to position the tubes in the centrifuge so to obtain a pellet on the back side of the tube, as described in the figure below (left).
3. Carefully and completely discard the supernatant as described in the figure below (right). Proceed to DNA extraction. If DNA extraction cannot be performed immediately, freeze the 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 obtain a pellet on the back side of the tube wall.



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 sample/product).

8.2 DNA extraction process

1.	Add 500 µl Lysis Buffer (transparent cap) to cell pellet. **Recommended for users of Microsart® ATMP Bacteria and Microsart® ATMP Fungi detection kit:** The Internal Control DNA, which is included in the detection kit can also be used to monitor the extraction process. Add 20 µl of 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 sec until the pellet is completely dissolved.
3.	Heat at 80 °C (preferably with shaking) for 10 min.
4.	Spin down at 16,200 × g for 10 min. **Attention:** Make sure to position the tubes in the rotor as indicated in the figure in chapter „Specimen“.
5.	Remove supernatant carefully and completely following the instructions given in chapter „Specimen“. Make sure not to disturb or aspirate the pellet in the process. **Attention:** There is a higher risk of PCR inhibition if liquid 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. 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, 100 CFU / vial, 3 vials each (Mollicutes)

SMB95-2051	Mycoplasma orale
SMB95-2052	Mycoplasma pneumoniae

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

SMB95-2003	Microsart® AMP Extraction (for mycoplasma)	50 extractions
SMB95-4000	Microsart® Proteinase K	50 extractions

Cleaning Spray

SMB95-5001	DNA Decontamination Reagent, spray bottle	250 ml
SMB95-5002	DNA Decontamination Reagent, refill canister	5 l

Cleaning Wipes

SMB95-5003	DNA Decontamination Reagent, wipes	50 wipes
SMB95-5004	DNA Decontamination Reagent, refill sachets	5 × 50 wipes

Notes

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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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)

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

Remove supernatant carefully

+ 100 µl Suspension Buffer (violet cap)

≥ 30 sec vigorously

DNA ready for PCR

incubate
vortex
centrifuge
add

storage at +18 - +25 °C
(ambient temperature)

Optional:
Add Internal Control from Microsart® ATMP Bacteria or Microsart® ATMP Fungi

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