Instructions for Use

Microsart® Geneprep

Concentration and DNA Extraction Kit for Liquids ≥ 100 ml

Prod. No. SMB95-2004
Reagents for 20 filtrations and DNA extractions
For use in research and quality control

85037-553-33

Contents

1 Intended Use

The Microsart® Geneprep kit can be used for isolation of genomic DNA from liquid samples. The kit was especially designed for qPCR assays to provide best performance in means of sensitivity and robustness for detection of bacterial genomic DNA within the test sample.

2 Test Principle

The flexibility of the test system allows a variety of water sources to be tested. Microsart® Geneprep utilizes 0.4 µm polycarbonate membranes for membrane filtration. After filtration the Microsart®@solve device is used for picking up the membrane from the funnel and dissolving the membrane filter with all retained microorganisms. This way it is an almost closed system with a minimized risk of contamination. Lysis buffer is added to the sample. After cell lysis is completed DNA isolation is done by a highly effective DNA precipitation protocol. The isolated DNA is ready to use for quantitative Real-time PCR analysis. Alternatively other DNA-based assays can be used.

3 Reagents and Disposables

Each kit contains reagents for 20 filtrations and DNA extractions. The kit components can be stored at room temperature (18 to 25°C). Carrier RNA that already has been rehydrated should be stored in aliquots at –18°C.

Kit Component	**Quantity**
Label Information	20 extractions Order No. SMB95-2004
Microsart®@filter	20
Microsart®@solve	20
Membrane Solvent	20 × 800 µl (Glass Vials) (blue cap)
Lysis Buffer	20 × 500 µl (Plastic Tubes) (yellow cap)
Separation Paste	20 × prefilled in Plastic Tubes (green cap)
1.5 ml Reaction Tube	20
Carrier RNA	2 Plastic Tubes (lyophilized) (red cap)
RNA Rehydration Buffer	1 Plastic Tube (black cap)
Isopropanol	14 ml
70% Ethanol	14 ml
DNA Rehydration Solution	2 × 1.5 ml (Plastic Tubes) (white cap)
Manual (Instructions for use)	1

4 User-supplied equipment and material

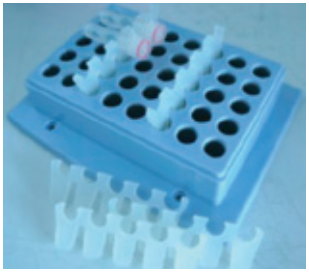

Figure 1: Reaction tube adapters

- PCR cycler (according the PCR kit requirements)
- PCR tubes (fitting to the PCR cycler)
- PCR kit
- Micropipettes and filter tips (10, 100 and 1000 µl)
- Vortex mixer
- Rack for 1.5/2 ml tubes
- Microsart® Combi.jet filtration manifold (Sartorius, Order No. 16848-CJ)
- Pump (e.g. Microsart® e.jet pump plus power supply and tubing, Sartorius; Order No. 166MP-4)
- Thermal shaker for 1.5/2 ml tubes
- Micro centrifuge for 1.5/2 ml tubes (e.g. Centrisart A14, Sartorius, Order No. A-14-1EU)
- At least 1 reaction tube adapter for 2 ml tubes for the thermal shaker, Sartorius; Order No. 1Z----0005 (5 tubes per adapter; see Figure 1; custom adapters can be supplied)
- At least two centrifuge adapters are necessary. We offer two different versions to be ordered: the adapter 1Z----0007 fits into the standard buckets for the circular 250ml bottles from Nalgene; the adapter Sigma 90672 fits into the rectangular buckets no. 13180 from Sigma. Alternatively custom products (adapters) can be supplied. Additionally a universal centrifuge incl. rotor is needed (Sartorius Centrifuge G-16 or G16C plus Rotor YCSR-S2B). Please contact us at PCR@Sartorius.com
- Please be advised that at least two centrifuge adapters are mandatory which can hold each one Microsart®@solve device (see technical drawings in Fig. 2, Fig. 3 and Fig. 4) and a 2 ml screw cap glass vial which is fixed at the outlet

Figure 2: Drawing, Lid and outlet part of the Microsart®@solve unit

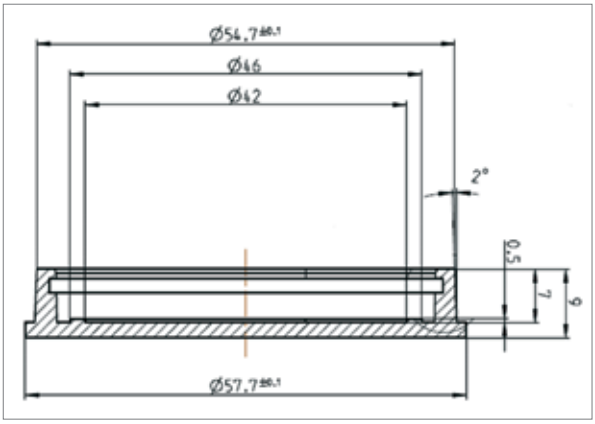

Figure 3: Technical drawing, lid of the Microsart®@solve unit

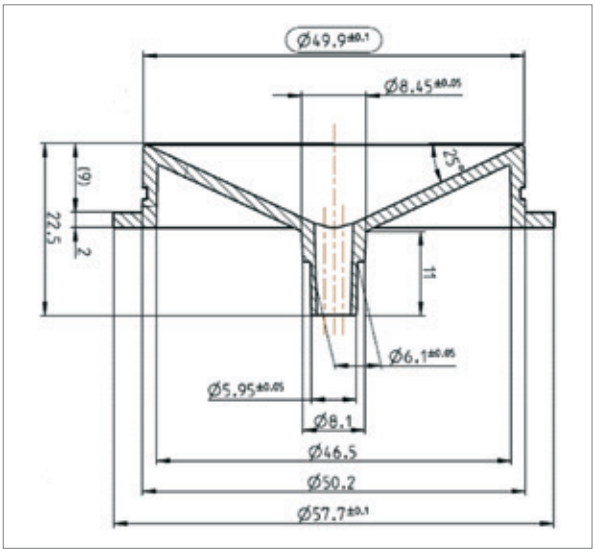

Figure 4: Technical drawing, outlet part of the Microsart®@solve unit

5 Precautions

The Microsart® Geneprep kit is intended for research use and quality control only, not for clinical diagnostics or testing of human samples without extensive validation. This kit should be used by trained persons only. All samples should be considered potentially infectious and handled according to local or national regulations. The kit substances may be disposed according to local regulations. Always wear a suitable lab coat, gloves and protective goggles.

The hazard and precautionary statements applying to the Membrane Solvent are:

H302	Harmful if swallowed.
H331	Toxic if inhaled.
H315	Causes skin irritation.
H319	Causes serious eye irritation.
H351	Suspected of causing cancer.
H361d	Suspected of damaging the unborn child.
H372	Causes damage to the kidneys and the liver through prolonged or repeated exposure.
P280	Wear protective gloves | protective clothing | eye protection | face protection.
P260	Do not breathe mist | vapors | spray.
P302+P352	If on skin: Wash with plenty of soap and water.
P308+P313	If exposed or concerned: Get medical advice | attention.

The hazard and precautionary statements applying to Isopropanol are:

H225	Highly flammable liquid and vapor.
H319	Causes serious eye irritation.
H336	May cause drowsiness or dizziness.
P210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P280	Wear protective clothing | eye protection.
P305+P351+P338	If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

The hazard and precautionary statements applying to 70% Ethanol are:

H225	Highly flammable liquid and vapor.
H319	Causes serious eye irritation.
P210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P233	Keep container tightly closed.
P305+P351+P338	If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

6 Procedure

Before starting each tube of Carrier RNA has to be rehydrated in 135 µl RNA Rehydration Buffer. Suitable aliquots should be prepared immediately and stored at –18°C until use. 10 µl Carrier RNA will be needed per sample.

Microsart®@filter and Microsart®@solve devices are single-use products which can be discarded after usage.

Please consider to prepare appropriate process negative and positive controls in parallel.

1. Membrane filtration of the sample using Microsart®@filter Polycarbonate (at least 100 ml should be filtered).
2. Remove the funnel → pick up the membrane filter with the help of the Microsart®@solve device (membrane will stick to the lid).

3. Connect lid and outlet part of the Microsart®@ solve via click fit mechanism.

4. Fix a glass vial (blue cap) at the outlet of the Microsart®@solve. The glass vial is prefilled with a membrane solvent.

5. The Microsart®@solve device is turned upside down and mixed a few seconds so that the solving reagent is transferred from the glass vial into the Microsart®@solve device. The membrane filter will be dissolved in only seconds.
6. The glass vial is removed from the Microsart®@ solve device (still turned upside down) and instead one plastic tubes containing Lysis buffer (yellow cap) is fixed at the outlet without spilling any Lysis buffer. Please keep the yellow cap for later use.

7. The Microsart®@solve incl. the attached tube is transferred into the suitable centrifuge adapter. The system is centrifuged one minute at 3.000 + g to transfer the dissolved membrane filter incl. all retained microbial cells into the attached tube with the Lysis buffer. The plastic tube is removed from the Microsart®@solve device and closed with the yellow screw cap again. Cell lysis occurs while mixing the tube horizontally on a thermal shaker thoroughly at 95°C for at least 15 minutes. The tubes should be fixed with the help of a tube adapter for thermal shakers. See chapter 4 of this manual.
8. The sample is transferred in the tube with the green cap which is prefilled with Separation Paste.
9. The tube is centrifuged 3 min at 16.000 × g for phase separation.
10. After the centrifugation step the paste separates the upper and the lower phase. The upper phase contains the DNA and can easily be transferred in an empty standard 1.5 ml reaction tube.
11. 600 µl of Isopropanol and 10 µl of rehydrated Carrier RNA (red cap) are added and inverted 50 times.
12. The tube is centrifuged 3 min at about 16.000 × g.
13. Discard the Isopropanol without losing the white pellet.
14. Add 600 µl of 70% Ethanol and invert 25 times (washing step).
15. The tube is centrifuged 1 min at 16.000 × g.
16. Discard the 70% Ethanol without losing the pellet.
17. The DNA pellet should be dried (5 to 10 min under a laminar flow for example).
18. The DNA pellet is rehydrated in 100 µl DNA Rehydration Solution (white cap). Rehydrate your samples in a Thermal shaker at 65°C while mixing (1 hour).
19. Your sample is ready for analysis via Real-time PCR or can be stored at –18°C or lower until PCR analysis.

7 Notes on the Procedure

1. This leaflet must be widely understood for a successful use of Microsart® Geneprep. The reagents supplied should not be mixed with reagents from different lots and used as an integral unit. The reagents of the kit should not be used beyond their shelf life.
2. Any deviation from the extraction method can affect the results.
3. For each setup, the use of control samples is advised to secure the day-to-day validity of results. Spiking with an Internal Amplification Control facilitates the evaluation of the extraction.
4. The DNA extract can be kept at –18°C or below for long term storage.
5. An even sample number has to be processed due to the high number of centrifugation steps during the procedure.

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The information and figures contained in these instructions correspond to the version date specified below.
Sartorius reserves the right to make changes to the technology, features, specifications and design of the equipment without notice.
Masculine or feminine forms are used to facilitate legibility in these instructions and always simultaneously denote the other gender as well.

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