Venor®GeM Sample Preparation Kit

Kit for the extraction of mycoplasma DNA from cell cultures and biopharmaceuticals for use with Venor®GeM Mycoplasma Detection Kits

INSTRUCTIONS FOR USE

FOR USE IN RESEARCH AND QUALITY CONTROL

Symbols

Lot No.

Cat. No.

Expiry date

Storage temperature

Number of reactions

Manufacturer

INDICATION

The Venor®GeM Sample Preparation Kit is intended for the isolation of mycoplasma DNA from cell culture or biopharmaceutical material. The isolated DNA can be used directly in combination with Mycoplasma Detection Kits from Minerva Biolabs for sensitive and robust mycoplasma detection with unprecedented performance.

This new version of Venor®GeM Sample Preparation Kit (product version 2) includes further developed components to achieve higher robustness for a wider range of samples. The instructions for use meet the criteria for EP-compliant (chapter 2.6.7) and JP-compliant (17th edition, chapter G3) testing. For isolating genomic DNA or total RNA from other organisms and sources such as eukaryotic tissues, bacteria, viruses, and peripheral blood, we recommend our ExtractNow kits. Please visit www.minerva-biolabs.com for further information.

PRINCIPLE OF THE METHOD

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, and (4) elution of purified DNA. The method does not require phenol/chloroform extraction and needs minimal hands-on time. The procedure is completed in ~30 minutes providing ready-to-use DNA for PCR.

CONTENT

Each kit contains reagents and components for 10, 50, or 200 extractions. The expiry date of the unopened package is marked on the package label. The kit components must be stored at room temperature (+15 to +25 °C).

Component	Quantity		
	10 Extractions Cat. No. 56-1010	**50 Extractions Cat. No. 56-1050**	**200 Extractions Cat. No. 56-1200**
Spin columns	10 units	50 units	4 x 50 units
Collection tubes	10 units	50 units	4 x 50 units
Conditioner	5 ml	15 ml	4 x 15 ml
Binding Buffer	10 ml	25 ml	4 x 25 ml
Buffer A1	3 ml (add 3 ml ethanol, abs., before first use)	15 ml (add 15 ml ethanol, abs., before first use)	4 x 15 ml (add 15 ml ethanol, abs., to each before first use)
Buffer A2	4 ml (add 16 ml ethanol, abs., before first use)	12 ml (add 48 ml ethanol, abs., before first use)	4 x 12 ml (add 48 ml ethanol, abs., to each before first use)
Buffer E	2 ml	2 x 2 ml	8 x 2 ml

The lot-specific quality control certificate (Certificate of Analysis) can be downloaded from our website (www.minerva-biolabs.com / www.minervabiolabs.us).

USER-SUPPLIED CONSUMABLES AND EQUIPMENT

The Venor®GeM Sample Preparation Kit contains reagents and components for isolating DNA from various sources. Additional consumables and equipment is supplied by the user:

- Ethanol > 96 % abs.
- 1.5 ml or 2 ml reaction tubes, DNA- and nucleases-free
- Microcentrifuge and heat block for 1.5 ml (or 2 ml) reaction tubes
- Pipettes with corresponding filter tips (100 and 1000 µl)
- Optional: Proteinase K (Cat. No. 56-0002) is needed for samples with high protein content (>10 mg/ml)
- Optional: Internal Control DNA "extra" is needed as spike-in to enable process controlling in combination with the Venor®GeM Classic Kit (e.g. Cat. No. 11-1025) or the Venor®GeM qEP Kit (e.g. Cat. No. 11-9025)

SPECIMEN

PCR inhibiting substances may accumulate over time in cell culture medium. Medium with more than 12 % serum has inhibitory effects on downstream application such as PCR. Moreover, phenol red, a standard ingredient in cell culture medium, might interfere with the optical read-out of fluorescence signals in qPCR. These adverse effects can be circumvented by using the Venor®GeM Sample Preparation Kit for DNA isolation and clean-up.

The Venor®GeM Sample Preparation Kit facilitates DNA isolation directly from cell culture supernatants containing up to 10^6 cells per ml.

Freshly collected cell culture materials should be extracted as soon as possible. Alternatively, cell culture materials can be stabilized by heat treatment (95 °C, 10 min, up to 500 µl sample volume) and stored for 1 week at room temperature.

Samples with high protein content (e.g. >10 mg/ml) may need to be treated with Proteinase K prior to DNA isolation (see protocol for further details).

Note that preparation of eukaryotic DNA from cells or tissues is not within the scope of the kit.

PRECAUTIONS

The Venor®GeM Sample Preparation Kit is intended for research use only. Clinical diagnostics or testing of human samples require extensive validation prior to use.

The kit should be used by trained laboratory staff only.

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

Always wear suitable lab coat and disposable gloves. The sample preparation waste contains Binding Buffer and Buffer A1, which may form highly reactive compounds when combined with bleaching agents. DO NOT add bleaching agents or acidic solutions directly to the sample preparation waste. Clean with suitable laboratory detergent and water, if any liquid is spilled.

The Binding Buffer contains propan-2-ol and polyethylene glycol octylphenol ether: flammable, harmful and irritant. The Buffer A1 contains guanidinium thiocyanate: harmful and irritant.

The hazard (H) statements according to the European Directive 1907/2006/EC (REACH) are listed below.

Component	Hazards	
Binding Buffer	H225	Highly flammable liquid and vapour.
	H336	May cause drowsiness or dizziness.
	H319	Causes serious eye irritation.
Buffer A1	H302	Harmful if swallowed.
	H314	Causes severe skin burns and eye damage.
	H412	Harmful to aquatic life with long lasting effects.
	H318	Causes serious eye damage.

Please see safety data sheets (SDS) on our website: www.minerva-biolabs.com for full information.

ADDITIONAL NOTES

⇒ These instructions must be understood to successfully use the Venor®GeM Sample Preparation 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 shelf life.

⇒ Any deviation from the extraction protocol may affect the results.

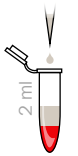
⇒ We recommend to include control samples on a regular basis to monitor the reliability of your results. This also proves advantageous in case of troubleshooting.

⇒ Do not use other alcohols other than ethanol as it will lead to inconsistent yields.

⇒ Pre-heating of Buffer E improves the yield significantly.

PROCEDURE - OVERVIEW

Isolation of Mycoplasma DNA



200 µl sample
200 µl Conditioner
10 sec

+ 70 °C for 10 min

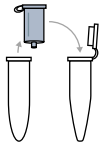
+ 400 µl Binding Buffer
10 sec

transfer all
11,000 × g
for 1 min
discard
flow-through

+ 500 µl Buffer A1
11,000 × g
for 1 min
discard
flow-through

+ 500 µl Buffer A2
11,000 × g
for 1 min
discard
flow-through

Full speed
for 3 min



change tube

+ 60 µl Buffer E
2 min

8000 × g
for 2 min

DNA ready for PCR

+ add
vortex
shake
incubate
centrifuge

MB_SI_Sample-Preparation-Kit_02_EN

PROCEDURE - STEP BY STEP

⇒ Before first use reconstitute Buffer A1 and Buffer A2 with absolute ethanol.

⇒ Set the heat block to 70 °C and equilibrate Buffer E at 70 °C.

1. Filtration

1.	Transfer up to **200 µl of cell culture material** into a new 1.5 ml reaction tube.
	For product release testing: add up to 50 µl of Internal Control DNA to the sample, e.g. add 30 µl or 12 µl of Internal Control DNA to each sample when using the Venor®GeM Classic Kit or the Venor®GeM qEP Kit, respectively.
2.	Add **200 µl of Conditioner,** vortex for at least 10 sec and incubate at 70 °C for 10 min. We recommend the use of a thermomixer for a permanent shaking of the sample. Alternatively, vortex the sample 3 to 4 times during the incubation. Equilibrate at room temperature for ~2 min before you proceed with step 3.
	Optional: add 10 µl Proteinase K per sample if the protein content is > 10 mg/ml. Vortex briefly and incubate as described above.
3.	Spin down the sample and add **400 µl of Binding Buffer** to the lysate. Vortex immediately and thoroughly in order to prevent any precipitation of nucleic acids. Do not centrifuge the sample and proceed immediately with the next step.
4.	Pipette the lysate into a spin column placed in a collection tube directly in the center of the spin column.
5.	Centrifuge the spin column at ≥ 10,000 x g for 1 min. Discard the flow-through from the collection tube and reassemble spin column and collection tube.
6.	Add **500 µl of Buffer A1**. Centrifuge the spin column at ≥ 10,000 x g for 1 min, discard the flow-through and reassemble the spin column and collection tube.
7.	Add **500 µl of Buffer A2**. Centrifuge the spin column at ≥ 10,000 x g for 1 min, discard the flow-through and reassemble the spin column and collection tube. Optional: Repeat the wash step with Buffer A2 once more.
8.	Centrifuge at full speed for 3 min in order to remove residual Buffer A2.
9.	Discard the collection tube and place the spin column into a new 1.5 ml reaction tube.
10.	Pipette **60 µl of pre-heated Buffer E** (70 °C) into the spin column directly onto the center of the silica membrane. The membrane's surface should be covered with the Buffer E.
11.	Incubate at room temperature for 2 min, then centrifuge at 8,000 x g for 2 min.
12.	The eluate contains the DNA and can be used directly for PCR or stored at +2 to +8 °C for a week. Long term storage should be at ≤-18 °C.

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 from the use, the results of use, or the inability to use this product.

Trademarks

Venor, Mynox, Onar and ZellShield are registered trademarks and 10CFU, 100CFU, PCR Clean, Mycoplasma Off, and WaterShield are trademarks of Minerva Biolabs GmbH, Germany.

ExtractNow™

The excellent way to isolate nucleic acids.
Kits for purifying nucleic acids from a variety of samples.
Find the optimized kit for your research needs.

ExtractNow™ Kit	Type of Sample	Package Size	Cat.-No.
DNA Mini Kit	• Tissue samples of up to 50 mg • Rodent tail specimens 0.5 – 1 cm in length • Paraffin samples (tissue) • Eukaryotic cells (max. 5 × 10^6) • Buccal swabs	10 extractions 50 extractions	601-1010 601-1050
Blood DNA Mini Kit	• Whole blood samples (up to 400 µl) • Fresh or frozen blood • Stabilizers: EDTA or citrate	10 extractions 50 extractions	602-1010 602-1050
RNA Mini Kit	• Eukaryotic cells (max. 5 × 10^6) • Tissue samples (max. 20 mg) • Gram+ and gram– bacteria (max. 1 × 10^9) • Biopsies	10 extractions 50 extractions	603-1010 603-1050
CleanUp Kit	• PCR reaction mixes (up to 50 µl) • TAE agarose gels (up to 300 mg) • TBE agarose gels (up to 300 mg)	10 extractions 50 extractions	604-1010 604-1050
Plasmid Mini Kit	• Bacterial suspensions • Isolation of high-copy plasmids: 0.5 – 5 ml • Isolation of low-copy plasmid DNA, P1 constructions, etc.: > 5 - 10 ml	10 extractions 50 extractions	605-1010 605-1050
Virus DNA/RNA Kit	• Serum, plasma, cell-free bodily fluids, supernatants from cell cultures (400 µl each) • Tissues and biopsies of up to 20 mg • Swab samples	10 extractions 50 extractions	606-1010 606-1050

Related Products

MB Taq DNA Polymerase

53-0050/-0100/-0200/-0250	MB Taq DNA Polymerase (5 U/µl)	50/100/200/250 units
53-1050/-1100/-1200/-1250	MB Taq DNA Polymerase (1 U/µl)	50/100/200/250 units

Contamination Control Kits for conventional PCR

11-1025/-1050/-1100/-1250	Venor®GeM Classic Mycoplasma Detection Kit	25/50/100/250 reactions
11-7024/-7048/-7096/-7240	Venor®GeM Advance Mycoplasma Detection Kit	24/48/96/240 reactions
11-8025/-8050/-8100/-8250	Venor®GeM OneStep Mycoplasma Detection Kit	25/50/100/250 reactions
12-1025/-1050/-1100/-1250	Onar® Bacteria Detection Kit	25/50/100/250 reactions

Contamination Control Kits for qPCR

11-9025/-9100/-9250	Venor®GeM qEP Mycoplasma Detection Kit	25/100/250 reactions
11-91025/-91100/-91250	Venor®GeM qOneStep Mycoplasma Detection Kit	25/100/250 reactions

Mycoplasma Elimination

10-0200/-0500/-1000	Mynox® Mycoplasma Elimination Reagent	2/5/10 treatments
10-0201/-0501/-1001	Mynox® Gold Mycoplasma Elimination Reagent	2/5/10 treatments

10CFU™ Sensitivity Standards, 3 vials with 10 CFU each, 2 vials negative control

102-1003	*Mycoplasma arginini*	
102-2003	*Mycoplasma orale*	
102-3003	*Mycoplasma gallisepticum*	
102-4003	*Mycoplasma pneumoniae*	
102-1103	*Mycoplasma salivarium*	
102-5003	*Mycoplasma synoviae*	
102-6003	*Mycoplasma fermentans*	
102-7003	*Mycoplasma hyorhinis*	
102-8003	*Acholeplasma laidlawi*i	
102-9003	*Spiroplasma citri*	
102-0002	Mycoplasma Set, all EP 2.6.7 listed species	2 vials per species, 10 CFU each

100CFU™ Sensitivity Standards, 3 vials with 100 CFU each, 2 vials negative control

103-1003	Mycoplasma arginini	
103-2003	Mycoplasma orale	
103-3003	Mycoplasma gallisepticum	
103-4003	Mycoplasma pneumoniae	
103-1103	Mycoplasma salivarium	
103-5003	Mycoplasma synoviae	
103-6003	Mycoplasma fermentans	
103-7003	Mycoplasma hyorhinis	
103-8003	Acholeplasma laidlawii	

PCR Clean™

15-2025/-2200/-2500	DNA Decontamination Reagent, Spray bottle/refill bottles	250 ml/4 × 500 ml/5 l
15-2001	DNA Decontamination Reagent, Wipes in a dispenser box	50 wipes
15-2002	DNA Decontamination Reagent, Wipes in refill bags	5 × 50 wipes

Mycoplasma Off™

15-1000/-5000	Surface Disinfectant, Spray bottle/refill bottles	1 l/5 l
15-1001	Surface Disinfectant, Wipes in a dispenser box	50 wipes
15-5001	Surface Disinfectant, Wipes in refill bags	5 × 50 wipes

ZellShield®

13-0050/-0150	Contamination Prevention Reagent 100x concentrate	50 ml/ 3 x 50 ml

WaterShield™

15-3015/-3020/-3050	Water Disinfection Additive for incubators and water baths, 200x concentrate	15 × 10 ml/3 × 50 ml/500 ml

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