Venor®GeM Sample Preparation Kit

Manual Extraction of Mycoplasma DNA from cell culture material and biopharmaceutical materials for use with Venor®GeM Mycoplasma Detection Kits

INSTRUCTIONS FOR USE

FOR USE IN RESEARCH AND QUALITY CONTROL

Symbols

	Lot No.
	Order No.
	Expiry date
	Storage temperature
	Number of extractions
	Manufacturer

INDICATION

The *Venor®GeM Sample Preparation* kit is developed for isolating mycoplasma DNA from cell culture or biopharmaceutical material. The isolated DNA in combination with Mycoplasma detection kits from Minerva Biolabs GmbH can be used directly for sensitive and robust detection of mycoplasma providing unprecedented performance.

The kit's chemistry is based on the former *MB DNA Extraction* kit but the instructions for use is adapted to meet the criteria for EP-compliant testing according to chapter 2.6.7.

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 go to: www.minerva-biolabs.com for further information.

PRINCIPLE OF THE METHOD

Cells are lysed by a combination of detergent and chaotropic salt. The lysate is directly applied onto a spin column, where DNA is selectively bound to highly specific silica membrane. Two subsequent washes remove residual contaminants, like proteins, metabolites, dyes, detergent etc. The purified DNA is eluted in Tris buffer. The procedure is completed in ~30 minutes providing DNA ready-to-use for PCR.

REAGENTS

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

Kit component	10 Preparations 56-1010	50 Preparations 56-1050	200 Preparations 56-1200
Spin columns	10 units	50 units	200 units
Collection tubes	10 units	50 units	200 units
Conditioner	2 x 1.5 ml	15 ml	50 ml
Buffer A1	2.4 ml (add 3.1 ml ethanol, abs., before first use)	11.5 ml (add 15 ml ethanol, abs., before first use)	45.8 ml (add 60.2 ml ethanol, abs., before first use)
Buffer A2	1.6 ml (add 3.8 ml ethanol, abs., before first use)	8 ml (add 18.6 ml ethanol, abs., before first use)	2 x 15.9 ml (add each 37.1 ml ethanol, abs., before first use)
Buffer E	1 ml	6 ml	14 ml

The lot-specific QC certificate (*Certificate of Analysis*) can be downloaded from our website (www.minerva-biolabs.com).

USER-SUPPLIED CONSUMABLES AND EQUIPMENT

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

- Ethanol > 96 % abs.
- Reaction tubes (1.5 ml)
- Microcentrifuge and heat block for 1.5 ml reaction tubes
- Pipettes with corresponding filter tips (100 and 1000 µl)
- Proteinase K (order No: 56-0002) is needed for samples with high protein content (>10mg/ml)
- *Internal Control DNA "extra"* is needed as spike-in to enable process controlling in conjunction with the *Venor®GeM Classic* kit (e.g. order No: 11-1025) or the *Venor®GeM qEP* kit (e.g. order 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, is likely to cross-react and thus falsifying 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 supernatant containing up to 10^6 cells per ml.

Untreated cell culture materials should be extracted as soon as possible. Cell culture materials can be stabilized by heat treatment (95 °C, 10 min, up to 500 µl) for 1 week at room temperature.

Samples with high protein content of >10mg/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 tissue is not within the scope of the kit.

PRECAUTIONS

The *Venor®GeM Sample Preparation* kit is intended for research use. 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 *Conditioner* 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 spilt.

The *Conditioner* and *Buffer A1* contain guanidine hydrochlorid: harmful and irritant.

The risk (R) and safety (S) phrases according to the European Directives 67/548/EEC and 1999/45/EC are:

R22	Harmful if swallowed
R36/37/38	Irritating to eyes, respiratory system and skin
R 42	May cause sensitization by inhalation
S 13	Keep away from food, drink and animal feeding stuffs
S 26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S 36	Wear suitable protective clothing.
S 46	If swallowed, seek medical advice immediately and show container or label.

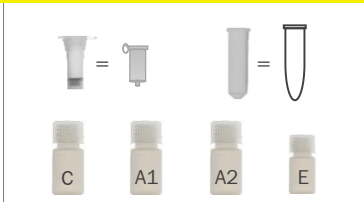
The risk phrases for *Conditioner* and *Buffer A1* according to EG 1272/2008 (GHS) are:

Acute Tox. 4, H302; Skin Irrit. 2, H315; Eye Irrit. 2, H319

This kit can be disposed of as municipal waste according to local guidelines.

Venor®GeM Sample Preparation Kit

Included



Duration

~ 30 min

Additionally required

- Ethanol > 96 % abs.
- 1.5 ml Reaction tubes
- Tools: Microcentrifuge
  Heat block
  Vortexer
  Pipettes + tips

Optional: Internal Control DNA „extra“
Proteinase K

Before first use!

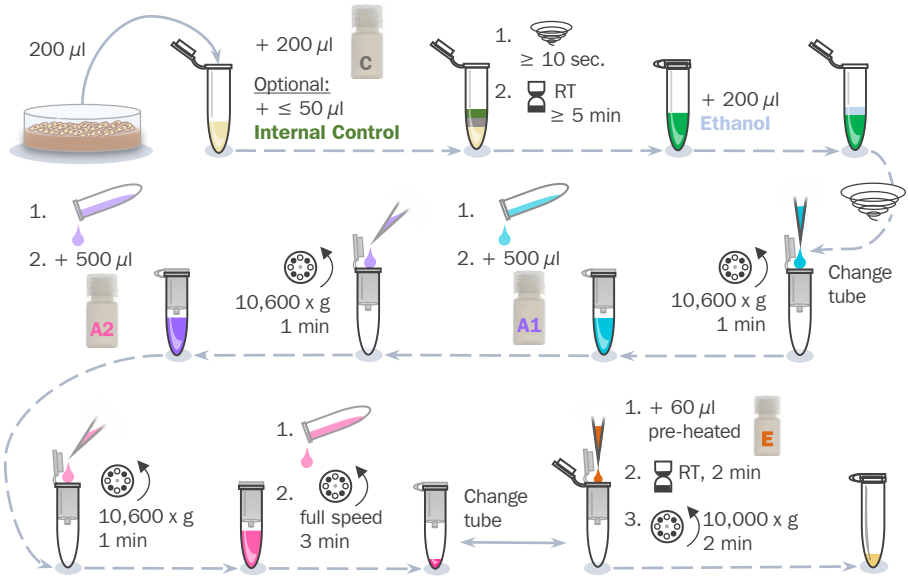
1 A1 + Ethanol > 96 % abs.

2 A2 + Ethanol > 96 % abs.

Preparation

E 70 °C

Procedure



Storage

- Store kit components at room temperature (18-25 °C). The expiry date of the unopened package is marked on the package label.
- Store purified DNA for 1 week at +2-8 °C or at -18 °C for long term storage.

Legend

Cell suspension	Sample	Heat
Conditioner	Buffer A1	Vortex
Internal Control	Buffer A2	Incubate
Proteinase K	Buffer E	Centrifuge
Ethanol	Purified DNA	

PROCEDURE - STEP BY STEP

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

⇒ Pre-heat *Buffer E* to 70 °C.

	Transfer up to **200 µl of cell culture material** into a new 1.5 ml reaction tube.
1.	*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 room temperature (18 to 25 ° C) for at least 5 min.
	Optional: add 10 µl Proteinase K per sample afterwards if the protein content is >10mg/ml. Vortex briefly and incubate at 56 °C for 15 min. Equilibrate at room temperature for ~2 min before you proceed with the next step.
3.	Add **200 µl of absolute ethanol** to the mixture. 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 sample lysate into a spin column placed in a collection tube without moistening the rim of the spin column.
5.	Centrifuge the spin column at ≥10,600 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 ≥10,600 x g for 1 min, discard the flow-through and reassemble the spin column and collection tube.
7.	Add **500 µl *Buffer A2***. Centrifuge the spin column at ≥10,600 x g for 1 min, discard the flow-through and reassemble the spin column and collection tube.
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 sample storage 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 ≥10,000 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.

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 LOT 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. It is also advantageous in case of troubleshooting.

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

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

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.

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 PCR Kits

11-1025/1050/1100/1250	Venor®GeM Classic Mycoplasma Detection Kit	25/50/100/250 tests
11-7024/7048/7096/7240	Venor®GeM Advance Mycoplasma Detection Kit	24/48/96/240 tests
11-8025/8050/8100/8250	Venor®GeM OneStep Mycoplasma Detection Kit	25/50/100/250 tests
12-1025/1050/1100/1250	Onar® Bacteria Detection Kit	25/50/100/250 tests
11-9025/9100/9250	Venor®GeM qEP Mycoplasma Detection Kit	25/100/250 tests

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

PCR Quantification Standards, 1x10^8 genomes / vial

52-0112	*Mycoplasma orale*
52-0115	*Mycoplasma gallisepticum*
52-0116	*Acholeplasma laidlawii*
52-0117	*Mycoplasma fermentans*
52-0119	*Mycoplasma pneumonia*
52-0124	*Mycoplasma synoviae*
52-0129	*Mycoplasma arginini*
52-0130	*Mycoplasma hyorhinis*
52-0164	*Spiroplasma citri*

See Minerva homepage for further available species

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*	
102-5003	*Mycoplasma synoviae*	
102-6003	*Mycoplasma fermentans*	
102-7003	*Mycoplasma hyorhinis*	
102-8003	*Acholeplasma laidlawii*	
102-9003	*Spiroplasma citri*	
102-0002	*Mycoplasma* Set, all EP 2.6.7 listed species	2 vials per species, 10 CFU each

PCR Clean™

15-2025/2200	DNA Decontamination Reagent, spray bottle/refill bottles	250 ml/4x 500 ml
15-2201	Wipes	120 wipes in a dispenser box
15-2202	Wipes, refill packs	5 x 120 wipes in a bag
15-2203	Wipes, single wrapped	30 wipes

Mycoplasma Off

15-1000	Surface Disinfectant Spray, spray bottle	1000 ml
15-5000	Surface Disinfectant Spray, refill bottles	5 x 1000 ml
15-1001	Surface Disinfectant Wipes in dispenser box	120 wipes
15-5001	Surface Disinfectant Wipes, refill pack	5 x 120 wipes
15-1030	Wipes, single wrapped	30 sachets

ZellShield™

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

WaterShield™

15-3025/3075	Water Disinfection Additive for incubators and water baths 200x concentrate	30 x 5 ml/500 ml

Manufacturer | Hersteller

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Ordering | Bestellung

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Made in Germany

Minerva Biolabs GmbH develops and manufactures products in accordance with DIN EN ISO 9001:2008 and DIN EN ISO 13485:2012 quality system requirement. Reg.No. SY 60096693 0001 & SX 60096692 0001

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