PCR Quantification Standard

Titrated Genomic DNA

INSTRUCTIONS FOR USE

FOR USE IN RESEARCH AND QUALITY CONTROL

Symbols

Lot No.

Order No.

Expiry date

Storage temperature

Number of reactions

Manufacturer

INTENDED USE

Our *PCR Quantification Standards* contain purified and adjusted genomic DNA of certain microorganisms to be used as either amplification and sensitivity control in conventional end-point PCR or in quantitative PCR as template material for generating standard curves with serial dilutions for PCR. DNA standard curves are commonly used to determine unknown DNA concentrations.

PRODUCT SPECIFICATIONS

Microorganisms were grown in liquid culture and harvested at the end of the logarithmic growth phase. The DNA is isolated by classical phenol-chloroform extraction followed by spin column purification. The DNA purity is assessed photometrically with a limit of OD260/280 ≥ 1.8. The microorganism's identity is confirmed by Sanger sequencing targeting specific regions in the genome.
The pure DNA extract is quantified by photometric analysis using a weighted calf thymus DNA standard. The quantification is confirmed by fluorometric analysis. The DNA is finally diluted and adjusted to 1 x 10^8 genome copies per vial. The dilution is confirmed by quantitative PCR analysis

REAGENTS

Each kit contains reagents for 10 dilution series. The expiry date of the unopened package is given on the package label.

Kit Component	Quantity	Cap Color
PCR Quantification Standard 1 x 10^8 genomes / vial	1 vial lyophilized	green
Tris Buffer 10 mM Tris, pH 8.5	3 x 2 ml	white

The kit components must be stored until use at +2 to +8 °C. The *PCR Quantification Standard* is lyophilized to sustain product stability. Thus the standard must be rehydrated before use. The rehydrated *PCR Quantification Standard* must be stored at < -18 °C. Freeze and thaw cycles are detrimental to DNA stability and must be avoided.

The LOT-specific *Certificate of Analysis* can be downloaded from our website: www.minerva-biolabs.com.

USER-SUPPLIED CONSUMABLES AND EQUIPMENT

The kit contains reagents for preparing 10 dilution series. Any other consumables and general laboratory equipment is supplied by the user:

- 1.5 ml reaction tubes, DNA- and RNA-free
- Pipettes with corresponding filter tips (10 and 100 µl)
- Microcentrifuge for 1.5 ml reaction tubes
- Vortexer

PRECAUTIONS

The kit is intended for research use only. Clinical diagnostics testing does require extensive validation.

This kit should be applied by experienced laboratory staff. Cross contamination may lead to false-positive results. The test should be performed according to good laboratory practice.

The kit does not contain hazardous substances. Remnants can be discarded according to local regulations.

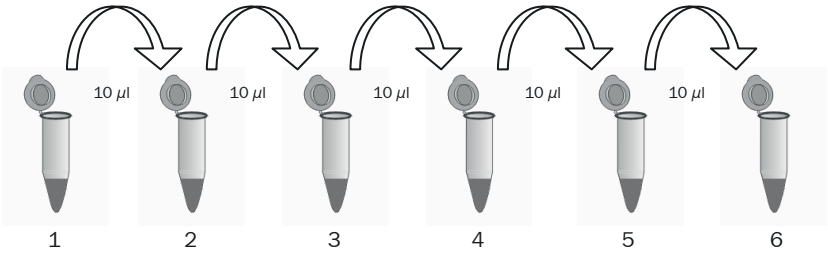
TEST PROCEDURE

1. Rehydration of the DNA

1.	Spin all vials briefly.
2.	Add 100 µl Tris Buffer to the vial (green cap).
3.	Incubate 5 min at room temperature.
4.	Vortex for 10 sec and spin for 5 sec.
5.	Use an appropriate volume of rehydrated DNA directly for PCR amplification (step 3) or proceed with dilutions for DNA standard curve (step 2).

2. Preparation of 10-fold dilutions for DNA standard curves

1.	Equilibrate rehydrated *PCR Quantification Standard* and Tris Buffer at room temperature.
2.	Label 1.5 ml reaction tubes consecutively. Pipett 90 µl Tris Buffer to each reaction tube.
3.	Vortex *PCR Quantification Standard* briefly and spin down.
4.	1st dilution: pipet 10 µl of the undiluted *PCR Quantification Standard* to the first reaction tube, close the tube and vortex briefly. Spin down briefly.
5.	2nd dilution: pipet 10 µl from the first dilution to the second reaction tube, close the tube and vortex briefly. Spin down briefly.
6.	3rd dilution: pipet 10 µl from the second dilution to the third reaction tube, close the tube and vortex briefly. Spin down briefly.
7.	Repeat theses steps for any additional reaction tube. A series of six dilutions is recommended.



3. PCR amplification

The rehydrated DNA (step 1) can be used directly for conventional PCR using a standard PCR assay of your choice. We recommend our Venor®GeM PCR kits for mycoplasma detection or our Onar® Bacteria PCR kit for bacteria detection.

For quantitative PCR use the serial dilutions (step 2) in order to generate a standard curve. The user may choose a suitable qPCR assay and cycler. However, the assay and cycler needs to be qualified. We recommend our qualified Venor®GeM qEP Mycoplasma Detection kit.

Most qPCR cycler control software allow quantitative analysis through a standard curve. This depends on a correct set-up including the standard curve specifications. Ensure that you define the standard curve samples in order to facilitate proper quantification. You may use the following specifications for your set-up. The volume used specifies the number of genome copies per PCR reaction:

Reaction tube no.	2 µl sample volume	5 µl sample volume	10 µl sample volume
1	$2x10^5$ genome copies	$5x10^5$ genome copies	$1x10^6$ genome copies
2	$2x10^4$ genome copies	$5x10^4$ genome copies	$1x10^5$ genome copies
3	$2x10^3$ genome copies	$5x10^3$ genome copies	$1x10^4$ genome copies
4	200 genome copies	500 genome copies	1000 genome copies
5	20 genome copies	50 genome copies	100 genome copies
6	2 genome copies	5 genome copies	10 genome copies

4. Evaluation

Using serial dilutions with established qPCR assays, the Ct-values will increase with descending DNA concentration. Generate a standard curve by means of Ct values and their quantities. The concentration of your unknown samples are calculated by interpolation based on the standard curve. The following amplification curves were generated with our Venor®GeM qEP Mycoplasma Detection Kit for qPCR on a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad Laboratories, Inc.).

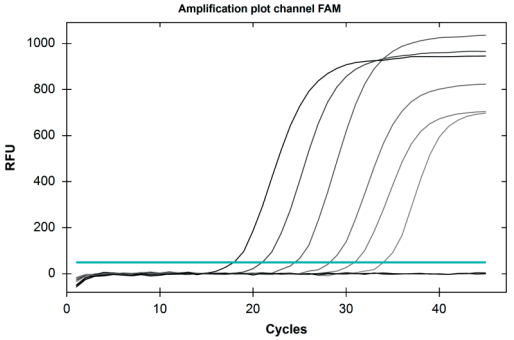


Fig. 1: Amplification curves from serial dilutions from 10^6 to 10 genome copies of *Mycoplasma fermentans*.

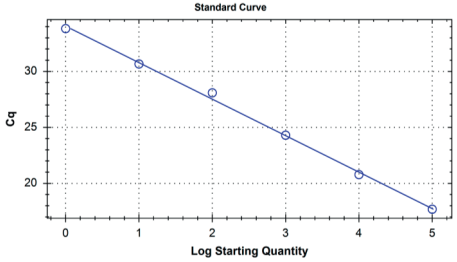


Fig. 2: Standard curve generated with the Bio-Rad CFX Manager software.

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

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Related Products

Contamination Control 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

Sample Preparation

56-1010/1050/1200	Venor®GeM Sample Preparation Kit	10/50/200 extractions
56-2096	Venor®GeM Sample Preparation Kit - IP C16	96 extractions

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

DNA Remover™

15-2025/15-2200	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 Sachets

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	1000 ml/ 5 x 1000 ml

WaterShield™

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

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