PCR Quantification Standard

Titrated Genomic DNA

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 PCR Quantification Standards contain pure, quantified genomic DNA of selected microbial species. This product is designed as a tool to assist quantitative PCR-based analysis.
Suitable for both conventional and real-time PCR, each PCR Quantification Standard can be applied to generate genomic DNA standard curves for specific microorganisms of interest, by serial dilution. In PCR-based methods, standard curves are essential to determine the unknown DNA concentration in a given sample. Additionally, the product can function as amplification, sensitivity, and specificity control in several PCR-based applications.

For the production of our PCR Quantification Standards, microorganisms are grown in liquid culture and harvested at the end of the logarithmic growth phase. From each culture, the DNA is isolated, quantified by fluorometric analysis and finally diluted to adjust for the final concentration (1×10^8 genome copies per vial).
The identity of each microorganism is confirmed by Sanger sequencing, targeting species-specific regions in the genome. The PCR Quantification Standard is lyophilized to enhance product stability.

REAGENTS

Each PCR Quantification Standard contains reagents for 10 dilution series for one particular microorganism. The expiry date of the unopened package is given on the package label. The components must be stored at +2 to +8 °C. Once rehydrated, the PCR Quantification Standard must be stored at ≤ -18 °C. Repeated freeze-thaw cycles must be avoided.

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

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

Each PCR Quantification Standard contains reagents for the preparation of 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
- Vortex

PRECAUTIONS

⇒ The PCR Quantification Standard is intended for research use only. Clinical diagnostics testing would require extensive validation.

⇒ The test should be performed according to good laboratory practice and should be applied by experienced laboratory staff.

⇒ PCR carry-over contamination may lead to false positive results.

⇒ The product does not contain hazardous substances. Remaining material can be discarded according to local regulations.

PROCEDURE – STEP BY STEP

1. Rehydration of the DNA (PCR Quantification Standard)

1.	Spin down the vials briefly.
2.	Add 100 µl Tris Buffer (white cap) to the PCR Quantification Standard vial (green cap).
	This rehydrated PCR Quantification Standard contains 10^6 genome copies (GC)/µl
3.	Incubate 5 min at room temperature.
4.	Vortex for 10 sec and spin down 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 standard curves

1.	Equilibrate the rehydrated PCR Quantification Standard and Tris Buffer at room temperature.
2.	Label 1.5 ml tubes with the corresponding standard concentration or, alternatively, with sequential numbers (see for example Fig. 1). Pipet 90 µl of Tris Buffer into each tube.
3.	Vortex PCR Quantification Standard for 10 sec and spin down for 5 sec.
4.	1st dilution: pipet 10 µl of the undiluted PCR Quantification Standard to the first tube (**1**), close, and vortex briefly. Spin down briefly.
5.	2nd dilution: pipet 10 µl from the first dilution to the second tube (**2**), close, and vortex briefly. Spin down briefly.
6.	3rd dilution: pipet 10 µl from the second dilution to the third tube (**3**), close, and vortex briefly. Spin down briefly.
7.	Repeat these steps for any additional tube. A series of six dilutions is recommended.

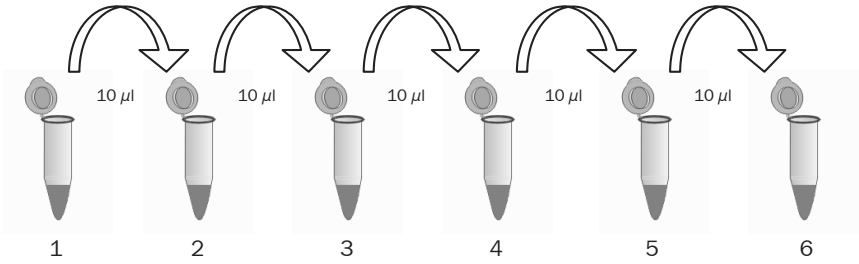

Fig. 1. Representative tubes and pipetting scheme for 6 serial dilutions of PCR Quantification Standard.

3. Amplification by PCR

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

For quantitative PCR, please use the serial dilutions indicated above (step 2.) to generate a standard curve. Any suitable qPCR assay and cycler may be selected. However, qualification of both the assay and the cycler is crucial for quantitative assays. We recommend for example our qualified Venor®GeM qEP Mycoplasma Detection kit.

Another essential issue here is the thorough and correct set-up of the samples and standards. Proper quantification of the samples depends on correct definition of the standard curve samples, since most qPCR cycler software allow automatic quantitative analysis through a standard curve.

Depending on the sample volume selected by the user for PCR, the following specifications (no. of genome copies, GC per PCR reaction) can be used for the serially diluted samples (labeled as in the PCR set-up):

Tube No. (Fig. 1)	Genome copies (Total/PCR reaction) for alternative PCR sample volumes:		
	2 µl	**5 µl**	**10 µl**
1	$2x10^5$	$5x10^5$	$1x10^6$
2	$2x10^4$	$5x10^4$	$1x10^5$
3	$2x10^3$	$5x10^3$	$1x10^4$
4	200	500	$1x10^3$
5	20	50	100
6	2	5	10

DATA INTERPRETATION

When using well established qPCR assays, the Ct values of serially diluted PCR Quantification Standards will inversely correlate with the DNA concentrations (i.e. increase with decreasing DNA concentrations). This is typically shown by the progressive rightward shift of the amplification plots for progressively less concentrated standards (Fig. 2). This inverse linear correlation is clearly represented by the obtained semi-log standard curves, where the Ct values appear on the Y-axis and the corresponding DNA quantities on the X-axis (log of genomic copies) (Fig. 3).

The concentrations of the unknown samples included in the assay can be calculated by simple interpolation along the standard curve, which is usually automatically done by the cycler software.

The linearity of the standard curve, as assessed by regression analysis and correlation coefficient (R^2), is essential for the reliability of the interpolated concentrations of the unknown samples.

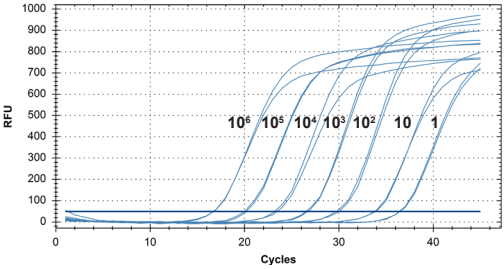

Fig. 2. Typical amplification plots of a serially diluted PCR Quantification Standard (Escherichia coli, 10^6 to 10 genome copies per μl, in duplicates).
qPCR was performed on CFX96 Touch™ from Bio-Rad Laboratories, Inc. RFU: relative fluorescence units.

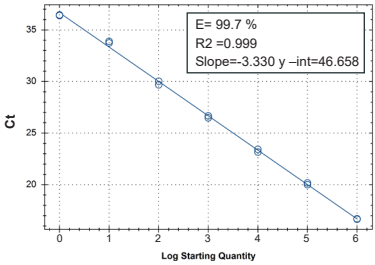

Fig. 3. Standard curve generated with data from Fig. 2 with the CFX Manager software (Bio-Rad Laboratories, Inc). Ct values appear on the Y-axis and the corresponding genomic copies per μl on the X-axis (in log). The R^2 and the slope of the standard curve as well as the amplification efficiency (E) are shown in the inset box.

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 100CFU, 10CFU, Cycler Check, PCR Clean, Mycoplasma Off, and WaterShield are trademarks of Minerva Biolabs GmbH. CFX Touch is a trademark of Bio-Rad Laboratories, Inc..

Related Products

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

Sample Preparation

56-1010/1050/1200	Venor®GeM Sample Preparation Kit	10/50/200 extractions
56-0002	Proteinase K	50 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, 10^8 genomes / vial

52-0116	*Acholeplasma laidlawii*
52-0129	*Mycoplasma arginini*
52-0117	*Mycoplasma fermentans*
52-0115	*Mycoplasma gallisepticum*
52-0130	*Mycoplasma hyorhinis*
52-0112	*Mycoplasma orale*
52-0119	*Mycoplasma pneumoniae*
52-0103	*Mycoplasma salivarium*
52-0124	*Mycoplasma synoviae*
52-0164	*Spiroplasma citri*
52-5571	*Bordetella pertussis*
52-0083	*Escherichia coli*
52-0101	*Legionella pneumophila*
52-0071	*Pseudomonas aeruginosa*

See MB homepage for further available species

Genomic DNA Extracts, 10 ± 2 ng / vial

51-0116	*Acholeplasma laidlawii*
51-0129	*Mycoplasma arginini*
51-0117	*Mycoplasma fermentans*
51-0115	*Mycoplasma gallisepticum*
51-0130	*Mycoplasma hyorhinis*
51-0112	*Mycoplasma orale*
51-0119	*Mycoplasma pneumonia*
51-0124	*Mycoplasma synoviae*
51-0164	*Spiroplasma citri*
2101-00819	*Aspergillus fumigatus*
51-0031	*Bacillus cereus*
51-0010	*Bacillus subtilis*
51-5571	*Bordetella pertussis*
51-1386	*Candida albicans*
51-7058	*Salmonella enterica*
51-0231	*Staphylococcus aureus*
51-0044	*Staphylococcus epidermidis*

See MB 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*
102-1103	*Mycoplasma salivarium*

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	

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*	
103-9003	*Spiroplasma citri*	

PCR Cycler Validation

57-2102	PCR Cycler Check™ Advance	6 strips, 8 vials each
57-2103	PCR Cycler Check™ OneStep	100 reactions
57-2202	qPCR Cycler Check™	100 reactions

SwabUp™ Lab Monitoring Kits

181-0010/-0050	Sample collection and DNA extraction	10/50 samples
182-0010/-0050	Sample collection, DNA extraction and PCR system	10/50 samples

AquaScreen® Detection kits for qPCR

33-2025/-2100/-2250	AquaScreen® Legionella species	25/100/250 reactions
34-2025/-2100/-2250	AquaScreen® Legionella pneumophila	25/100/250 reactions
34-6025/-6100/-6250	AquaScreen® Pseudomonas aeruginosa	25/100/250 reactions
34-7025/-7100/-7250	AquaScreen® Escherichia coli	25/100/250 reactions

PCR Clean™

15-2025/-2200	DNA Decontamination Reagent, spray bottle/refill canister	250 ml/4×500 ml
15-2500	DNA Decontamination Reagent, refill canister	5 l
15-2001	DNA Decontamination Reagent, Wipes in dispenser box	50 wipes
15-2002	DNA Decontamination Reagent, Wipes, refill pack	5×50 wipes

Mycoplasma Off™

15-1000/-5000	Surface Disinfectant Spray, spray bottle, refill canister	1 l/5 l
15-1001	Surface Disinfectant Wipes in dispenser box	50 wipes
15-5001	Surface Disinfectant Wipes in refill pack	5×50 wipes

ZellShield®

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

WaterShield™

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

Minerva Biolabs GmbH
Schkopauer Ring 13
D-12681 Berlin, Germany

www.minerva-biolabs.com
Ordering: order@minerva-biolabs.com
Support: support@minerva-biolabs.com

USA & Canada

Minerva Biolabs Inc.
1 Jill Ct., Building 16, Unit 10
Hillsborough, NJ 08844
USA

www.minervabiolabs.us
Ordering: order@minervabiolabs.us
Support: help@minervabiolabs.us

Made in Germany

HB3.07EN

minerva biolabs®