dPCR Validation Standard

Quantified genomic DNA from selected species or cell lines

INSTRUCTIONS FOR USE

FOR USE IN RESEARCH AND QUALITY CONTROL

SYMBOLS

Lot No.

Cat. No.

Expiry date

Storage temperature

Number of reactions

Manufacturer

INTENDED USE

The dPCR Validation Standard contains pure, quantified genomic DNA of the selected species or cell lines. This product is designed as a tool to assist residual DNA dPCR-based analysis.

Each dPCR Validation Standard can be applied to generate genomic DNA Standard curves for the specific species or cell lines of interest, by serial dilution. A standard curve can be used to convert the measured DNA in copies/µl through dPCR in fg/µl to provide a quantification of the residual DNA in conformity to regulatory guidelines.

In addition, the dPCR Validation Standards can be used during downstream processes to verify the residual DNA concentration of the processed sample during several purification processes.

CONTENT / REAGENTS

Each dPCR Validation Standard contains one vial with 1 ng of lyophilized genomic DNA and one vial of Tris Buffer. The expiry date of the unopened package is given on the package label. The components must be stored at +2 °C to +8 °C until use.
Once rehydrated, the dPCR Validation Standard can be stored at ≤-18 °C for a maximum of 30 days. Repeated freeze-thaw cycles must be avoided.

Components

Component	Quantity	Cap color
dPCR Validation Standard 1ng/vial	1 vial, lyophilized	green
Tris Buffer (10 mM TRIS-HCl, pH 8.5)	1 x 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 dPCR Validation 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 µl and 100 µl)
- Microcentrifuge for 1.5 ml reaction tubes
- Vortex

PRECAUTIONS

The dPCR Validation Standard is intended for use in research and quality control.
The test should be performed by trained laboratory staff only.
Always wear a suitable lab coat and disposable gloves. Remnants can be discarded according to local regulations.
Performing the tests according to good laboratory practice helps avoiding carry-over contaminations and false positive results and, ultimately, helps obtaining reliable results.

ADDITIONAL NOTES

These instructions must be followed exactly for the successful use of the dPCR Validation Standard. Any deviation may affect the test method and the results. The reagents supplied should not be mixed with reagents from different lots and used as an integral unit. The reagents of the kit must not be used beyond the expiry date.

PROCEDURE - STEP BY STEP

1. Rehydration of the DNA

1.	Spin down the vial briefly.
2.	Add 100 µl Tris Buffer (white cap) to the dPCR Validation Standard vial (green cap). The rehydrated dPCR Validation Standard contains 10000 fg/µl of genomic DNA.
3.	Incubate 5 min at room temperature.
4.	Vortex for 10 s and spin down for 5 s.
5.	Use an appropriate volume of rehydrated DNA directly for dPCR amplification (step 3.) or proceed with dilutions for the DNA standard curve (step 2.).

2. Preparation of 10-fold dilutions for the standard curve

1.	Equilibrate the rehydrated dPCR Validation Standard and the Tris Buffer at room temperature.
2.	Label 1.5 ml tubes with the corresponding standard concentration or, alternatively, with sequential numbers. Pipet 90 µl of Tris Buffer into each tube.
3.	Vortex the dPCR Validation Standard for 10 s and spin down for 5 s again.
4.	1st dilution: Pipet 10 µl of the undiluted PCR Validation Standard to the first tube, close, and vortex briefly. Spin down briefly (concentration: 1000 fg/µl).
5.	2nd dilution: Pipet 10 µl from the first dilution to the second tube, close, and vortex briefly. Spin down briefly (concentration: 100 fg/µl).

6.	3rd dilution: Pipet 10 µl from the second dilution to the third tube, close, and vortex briefly. Spin down briefly (concentration: 10 fg/µl).
7.	Repeat these steps for any additional tube. A series of four dilutions is recommended.

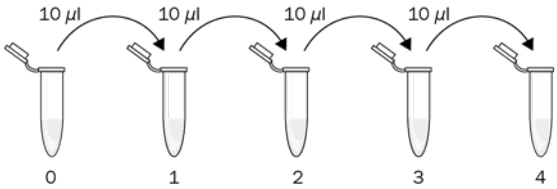

Fig. 1. Representative tubes and pipetting scheme for four serial dilutions of the dPCR Validation Standard

3. Amplification by dPCR

The rehydrated dPCR Validation Standard can be used directly for dPCR by means of MiQuant® Residual DNA dPCR Kits. Please note that the high concentration of the undiluted dPCR Validation Standard might lead, according to the volume of sample loaded, to high positive partitions coverage in the 24K Nanowell plate, therefore affecting the precision of the results. Depending on the sample volume of choice, the following specifications (copies/µl, fg/µl) can be used for the serially diluted samples:

Tube No.	fg/µl per reaction for alternative PCR sample volumes in 40 µl total reaction volume		
	2 µl	4 µl	10 µl
0 (10000 fg/µl)	500	1000	2500
1 (1000 fg/µl)	50	100	250
2 (100 fg/µl)	5	10	25
3 (10 fg/µl)	0.5	1	2.5
4 (1 fg/µl)	0.05	0.1	0.25

DATA INTERPRETATION

Fluorophore color	Exposure / Gain
Green (DNA Standard)	500 ms / 6
Yellow (Internal Control)	500 ms / 6

It is recommended to use the automatic threshold settings for the analysis. In exceptional cases (e.g. testing an inhibitory matrix that might cause increased „rain“), the setting of a manual threshold might be required.

Suggestion: In case of manual threshold setting, it is recommended to set the same threshold throughout all samples of the same experiment by choosing a cut-off fluorescence value that divides best positive partitions from negative partitions.

Conversion from copies/µl to fg/µl

Method 1: Standard curve

1.	Run a ten-fold serial dilution of the DNA Standard (e.g 40000 fg/reaction to 4 fg/reaction = 1000 to 0.1 fg/µl in 40 µl total reaction volume).
2.	Perform a linear regression of the obtained data from the standard curve to determine its equation like below: **y=mx-q** whereby: x = known concentration of the DNA-standard at a given dilution in pg/µl y = measured concentration of the DNA-standard at the given dilution in copies/µl m = slope of the standard curve q = intercept of the standard curve
3.	Convert the equation to x to determine the concentration of the unknown sample in pg/µl **x=(y+q)/m** whereby: y = measured concentration of the unknown sample in copies/µl x = concentration of the unknown sample in pg/µl m = slope of the standard curve q = intercept of the standard curve
4.	Obtain the concentration of the unknown sample in pg/µl by substituting the y in the equation with the measured concentration of the sample in cp/µl.

Method 2: Calculate a conversion factor

1.	From the data obtained from the standard curve, it is possible to determine a conversion factor for your matrix and your experimental conditions for future use. The conversion factor is calculated by dividing y/x whereby: y = known concentration of the DNA-standard at a given dilution in pg/µl x = measured concentration of the DNA-standard at the given dilution in copies/µl
2.	This can be done by calculating the conversion factor of one data point of the DNA-standard or taken the conversion factor of all data points of the DNA-standard curve into the equation and determine the average conversion factor of all data points.
3.	The experimental obtained copies/µl of the sample are multiplied by the conversion factor.
4.	The obtained conversion factor can be used for future validations to converse copies/µl to pg/µl of the sample if the matrix, in which the sample is diluted, is unaltered. When there are changes to the composition of the matrix, the conversion factor must be calculated again by running a standard curve or at least determine the conversion factor from one data point of a given concentration.

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

QIAcuity is a registered trademark of QIAGEN NV, Netherlands. MiQuant, Venor, Mynox and ZellShield are registered trademarks and PCR Clean, Mycoplasma Off and WaterShield are trademarks of Minerva Biolabs GmbH, Germany.

RELATED PRODUCTS

Residual DNA Detection Kits for the QIAGEN digital PCR system		
58-0101/-0102	MiQuant® Residual DNA dPCR E. coli	24/4×24 reactions
58-0111/-0112	MiQuant® Residual DNA dPCR CHO	24/4×24 reactions
58-0121/-0122	MiQuant® Residual DNA dPCR HEK-293	24/4×24 reactions
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 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	15×10 ml/3×50 ml/500 ml 200× concentrate

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.
400 Route 518 Skillman,
NJ 08558, USA

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

Made in Germany

minerva biolabs®