MiQuant® Residual DNA dPCR CHO

Detection of residual DNA from CHO cells using the QIAGEN digital PCR system

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 MiQuant® Residual DNA dPCR CHO is designed for the quantitative detection of residual DNA from CHO (Chinese Hamster Ovary) cells in chemical and biological matrices.

The MiQuant® Residual DNA dPCR CHO is designed for use with the QIAGEN QIAcuity digital PCR (dPCR) system as a novel, rapid, robust and sensitive method for the detection of residual host cell DNA.

PRINCIPLE OF THE METHOD / TEST PRINCIPLE

Notably, dPCR is lesser sensitive to inhibitory factors in comparison to qPCR. Therefore, the kit offers the possibility of direct testing of samples without the need of DNA extraction. This extremely reduces the time needed for sample testing to less than 3 hours (including dPCR preparation and thermocycling) and increases the quality of DNA quantification as no DNA material is being lost during extraction.

The kit offers an extremely high flexibility to the customers because it is possible to load up to 20 μl of sample volume.

CHO residual DNA is detected by amplifying a short multicopy target in the genome. The length of the amplicon is short enough to allow the amplification of highly degraded DNA.

The kit contains all necessary PCR components including Taq polymerase, primers and dNTPS in a lyophilized mix as well as a Rehydration Buffer and dPCR grade Water. False negative results caused by PCR inhibition will be reliably identified by means of an internal control DNA, which is recommended to be added directly to the master mix. The amplification of the internal control DNA is detected in the yellow channel, whereas the amplification of CHO is detected in the green channel.

CONTENT / REAGENTS

Each kit contains reagents for 24 or 96 reactions. The expiry date of the unopened package is marked on the package label. The kit components must be stored at +2 °C to +8 °C until use. The rehydrated components can be stored at ≤ -18 °C for a maximum of 30 days.

Components

Component	Quantity		Cap color
	24 reactions Cat. No. 58-0111	**4 x 24 reactions Cat. No. 58-0112**	
MiQuant® CHO Mix	1 vial lyophilized	4 vials lyophilized	red
Rehydration Buffer	1 vial 550 µl	4 vials with 550 µl each	blue
Positive Control DNA	1 vial lyophilized	4 vials lyophilized	green
Internal Control DNA	1 vial lyophilized	4 vials lyophilized	yellow
dPCR grade Water	1 vial 2 ml	4 vials with 2 ml each	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

The MiQuant® Residual DNA dPCR CHO kit contains PCR reagents for the specific detection of residual DNA from CHO cells. PCR consumables and equipment are supplied by the user:

- QIAcuity Digital PCR System
- QIAcuity Nanoplates 26k 24-well
- PCR Clean (15-2025, 15-2001)
- PCR reaction tubes or plates
- Microcentrifuge for PCR reaction tubes

SPECIMEN / SAMPLES

Direct testing, no extration required.

PRECAUTIONS

The MiQuant® Residual DNA dPCR CHO kit is for use in research and quality control.
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 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 to successfully use the MiQuant® Residual DNA dPCR CHO kit. 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. Reagent preparation

The test should be carried out with negative and positive controls and samples in duplicates.
The rehydrated components can be stored at ≤ -18 °C for a maximum of 30 days.
It is recommended to avoid repeated freezing/thawing cycles and to store rehydrated controls (Internal Control and Positive Control) in aliquots.

1.	MiQuant® CHO Mix Internal Control DNA Positive Control DNA	Red cap Yellow cap Green cap	Spin down all lyophilized components at max speed for 5 sec.
2.	MiQuant® CHO Mix	Red cap	Add 530 µl Rehydration Buffer (blue cap).
3.	Internal Control DNA	Yellow cap	Add 1000 µl dPCR grade Water (white cap).
4.	Positive Control DNA	Green cap	Add 100 µl dPCR grade Water (white cap).
5.	MiQuant® CHO Mix Internal Control DNA Positive Control DNA	Red cap Yellow cap Green cap	Incubate at RT for 5 min.
6.	MiQuant® CHO Mix Internal Control DNA Positive Control DNA	Red cap Yellow cap Green cap	Vortex and spin down for 5 sec.

2. Reaction mix preparation

Prepare the required volume of master mix for the number of samples and add the required amount of internal control into the mix.

	Component	Cap Colour	for 1 reaction	for 24 reactions
1.	MiQuant® CHO Mix	Red	20 µl	480 µl
	Internal Control DNA	Yellow	1 µl	24 µl
2.	Homogenize the master mix by pipetting.			
3.	Pipet 20 µl of mix to each tube, discard the remaining material.			

3. Add samples

Set up negative (no template controls, NTCs), and positive controls in each PCR.

1.	NTCs: Add 20 µl of matrix or dPCR grade Water.
2.	Test samples: Add a variable volume of test sample between 4 µl and 20 µl and respective amount of dPCR grade Water to reach a total volume of 40 µl / sample.
3.	Positive Control: Add 4 µl / well and 16 µl of dPCR grade Water.

Spin PCR tubes briefly and transfer 38 µl of each sample to each well of the QIAcuity Nanoplate. It is very important to prevent the formation of bubbles in the well for an efficient partitioning of the sample through the nanowells.

4. Start PCR amplification

1. Seal the nanoplate according to manufacturer‘s instruction.

2. Program the QIAcuity as follows:

Priming: QIAGEN standard priming profile.
Cycling:

Step	Time	Temperature (°C)	Cycles
PCR initial heat activation	2 min	95	1
Denaturation	15 s	95	5
Combined annealing/extension	30 s	60	
Denaturation	15 s	95	40
Combined annealing/extension	30 s	66	

2.	**Imaging:**	
	Fluorophore	**Exposure / Gain**
	Green (CHO)	500 ms / 6
	Yellow (Internal control)	500 ms / 6
3.	Start the program.	

DATA INTERPRETATION

The presence of CHO residual DNA is indicated by fluorescence in the green channel and the internal control signal is displayed in the yellow channel. The quantification is calculated as copies/µl per reaction.

A positive dPCR result for CHO is indicated when positive partitions in the tested well are observed. Due to the high sensitivity of the kit, it is advised to clean working benches and racks thoroughly with PCR Clean™ before each experiment to minimise contaminations with CHO DNA.

ASSAY CHARACTERISTICS

Sensitivity

The required quantification limit of *European Pharmacopoeia* 2.6.35 is 10 fg/µl in the sample to be tested. The kit showed during our validation study a sensitivity of 0.33 fg/µl per reaction with a CV of 15.01% and a limit of detection of 0.04 fg/µl per reaction by means of dPCR Validation Standard CHO.

Specificity

No relevant unknown cross reactivity was found *in vitro* with indicated unrelated species (HEK-293, *Pichia pastoris, Bacillus subtilis, E.coli*, HT1080). Cross reactivity for *Mus musculus* was observed due to the high phylogenetic similarity between the two species.

APPENDIX

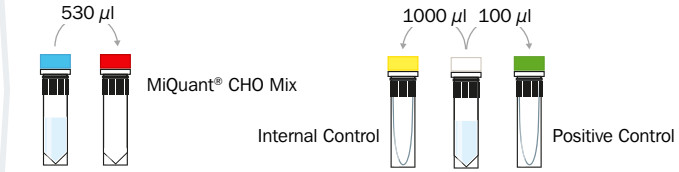
Limited Product Warranty

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.

PROCEDURE – OVERVIEW

1. Reagent preparation

- MiQuant® CHO Mix
- Internal Control DNA
- Positive Control DNA



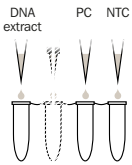
5 min RT
briefly
for 5 sec

2. Reaction mix preparation

Reaction mix for 1 reaction
20 µl MiQuant® CHO Mix
1 µl Internal Control DNA

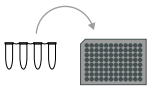
3. Add samples

20 µl reaction mix
+ a variable volume of test sample between 4 µl and 20 µl and respective amount of dPCR grade Water to reach a total volume of 40 µl / sample
+ or
4 µl Positive Control and 16 µl dPCR grade Water
+ or
20 µl of NTC

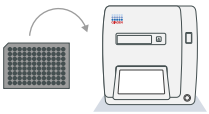


briefly

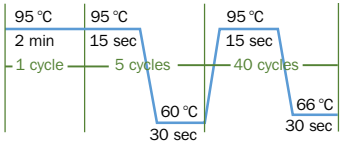
Transfer 38 µl of each sample to each well of the QIAcuity Nanoplate



4. Start PCR amplification



Start dPCR program



Imaging:

Fluorophore	Exp. /Gain
Green (CHO)	500 ms / 6
Yellow (IC)	500 ms / 6

Rehydration Buffer
MiQuant® CHO Mix
dPCR grade Water

Positive Control
Internal Control
centrifuge

+ add
incubate
vortex

NTC = No Template Control
PC = Positive Control
IC = Internal Control

This procedure overview is not a substitute for the detailed manual.

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Trademarks

QIAcuity is a registered trademark of QIAGEN NV, Netherlands. MiQuant, Venor, Mynox, Onar, and ZellShield are registered trademarks and PCR Clean, Mycoplasma Off, 10CFU, 100CFU, and WaterShield are trademarks of Minerva Biolabs GmbH, Germany.

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	25/100/250 reactions
12-1025/-1100/-1250	Onar® Bacteria Detection Kit	25/100/250 reactions

Contamination Control Kits for qPCR

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

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 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 Clean™

15-2025/-2200/-2500	Decontamination Reagent, spray bottle/refill bottles/canister	250 ml/4×500 ml/5 l
15-2001	Decontamination Reagent, Wipes in dispenser box	50 wipes
15-2002	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	15×10 ml/3×50 ml/ 500 ml
	and water baths	200× concentrate

DNA Extraction kits

56-1010/-1050/-1200	Venor®GeM Sample Preparation Kit	10/50/200 extractions
601-1010/-1050	ExtractNow™ DNA Mini kit	10/50 extractions
602-1010/-1050	ExtractNow™ Blood DNA Mini kiT	10/50 extractions
603-1010/-1050	ExtractNow™ RNA Mini kit	10/50 extractions
604-1010/-1050	ExtractNow™ Cleanup kit	10/50 extractions
605-1010/-1050	ExtractNow™ Plasmid Mini kit	10/50 extractions
606-1010/-1050	ExtractNow™ Virus DNA/RNA kit	10/50 extractions
607-1010/-1050	ExtractNow™ Vegan Control	10/50 extractions
608-1010/-1050	ExtractNow™ Meat ID	10/50 extractions
32-1010/-1050	AquaScreen® FastExtract	10/50 extractions

SwabUp™ Lab Monitoring Kits

181-0010/-0050	Sample collection and DNA extraction	10/50 samples

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Made in Germany

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