# MiQuant® Residual DNA dPCR E. coli

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 E. coli is designed for the quantitative detection of residual DNA from E. coli cells in chemical and biological matrices.

The MiQuant® Residual DNA dPCR E. coli 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 (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 $\mu$l of sample volume.

E. coli 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 E. coli 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. 58-0101 | 4 x 24 reactions Cat. No. 58-0102 | |
| MiQuant® E. coli 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 E. coli 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 E. coli 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 E. coli 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® E. coli Mix<br>Internal Control DNA<br>Positive Control DNA | Red cap<br>Yellow cap<br>Green cap | Spin down all lyophilized components at max speed for 5 sec. |
| 2. | MiQuant® E. coli 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® E. coli Mix<br>Internal Control DNA<br>Positive Control DNA | Red cap<br>Yellow cap<br>Green cap | Incubate at RT for 5 min. |
| 6. | MiQuant® E. coli Mix<br>Internal Control DNA<br>Positive Control DNA | Red cap<br>Yellow cap<br>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® E. coli 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 | 45 |
| Annealing | 10 s | 55 | |
| Extension | 30 s | 60 | |

**Imaging:**

| Fluorophore | Exposure / Gain |
|---|---|
| Green (E. coli) | 500 ms / 6 |
| Yellow (Internal control) | 500 ms / 6 |

3. Start the program.

## DATA INTERPRETATION

The presence of *E. coli* 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 *E. coli* 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 *E. coli* DNA.

## ASSAY CHARACTERISTICS

### Sensitivity

The required quantification limit of the European Pharmacopoeia 2.6.35 is 10 fg/µl for the sample to be tested. The kit showed during our validation study a sensitivity of 0.5 fg/µl per reaction with a CV of 19.3% by means of the dPCR Validation Standard E. coli.

### Specificity

No relevant unknown cross reactivity was found in vitro with indicated unrelates species (HEK-293, *Pichia pastoris*, *Bacillus subtilis*, CHO, *Pseudomonas aeroginosa*). Cross reactivity for *Shigella flexneri* was observed due to the high phylogenetic similarity between the two species.

## APPENDIX

*Limited Product Warranty*

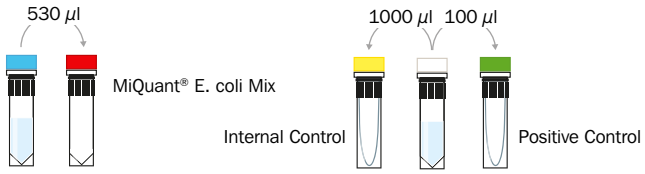
This warranty limits our liability for replacement of this product. No warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided. Minerva Biolabs shall have no liability for any direct, indirect, consequential, or incidental damages arising from the use, the results of use, or the inability to use this product.

*Trademarks*

## PROCEDURE – OVERVIEW

### 1. Reagent preparation

MiQuant® E. coli Mix
Internal Control DNA
Positive Control DNA



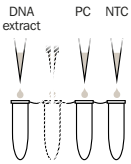

5 min RT
briefly
for 5 sec

### 2. Reaction mix preparation

**Reaction mix for 1 reaction**
20 µl MiQuant® E. coli Mix
1 µl Internal Control DNA

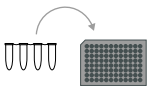
### 3. Add samples

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

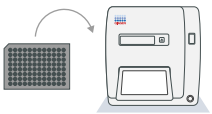



briefly

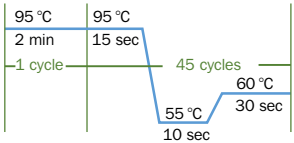
Transfer 38 µl of each sample to each well of the QIAcuity Nanoplate



### 4. Start PCR amplification



Start dPCR program




Imaging:

| Fluorophore | Exp./Gain |
|---|---|
| Green (*E. coli*) | 500 ms / 6 |
| Yellow (IC) | 500 ms / 6 |

Rehydration Buffer
MiQuant® E. coli 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.

MB_SI_58.01.01EN

## 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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