March 2024

# dPCR Copy Number Variation (CNV) Probe Assays Handbook

For quantification of gene-specific regions in human, mouse, or rat DNA using dPCR CNV Probe Assays on the QIAcuity® platform

Sample to Insight

# Contents

# Kit Contents

dPCR CNV Probe Assays for more than 200 human genes of interest, 4 human reference genes, and 24 human centromeric reference genes have been wet-lab validated. If users wish to quantify gene targets for which no wet-lab validated assays exist, custom dPCR CNV Probe Assays for targets in human, mouse, and rat can be designed and ordered at QIAGEN GeneGlobe.

## Wet-lab Validated dPCR CNV Probe Assays

**Table 1. Wet-lab validated dPCR CNV Probe Gene of Interest Assay (cat. no. 250210)**

| Detection Channel | Dye | Size* | GeneGlobe ID |
|---|---|---|---|
| Green | **FAM** | 300 rxns | DCG########-F300 |
| Green | **FAM** | 1000 rxns | DCG########-F1000 |
| Orange | **ATTO 550** | 500 rxns | DCG########-A500 |
| Crimson | **Cy5** | 500 rxns | DCG########-C500 |

* Number of reactions are based on 12 µL reaction volumes in Nanoplate 8.5k.

**Table 2. Wet-lab validated dPCR CNV Probe Reference Assay (cat. no. 250212)**

| Detection Channel | Dye | Size* | GeneGlobe ID |
|---|---|---|---|
| Yellow | **HEX** | 300 rxns | DCR########-H300 |
| Yellow | **HEX** | 1000 rxns | DCR########-H1000 |
| Red | **ROX** | 300 rxns | DCR########-R300 |
| Red | **ROX** | 1000 rxns | DCR########-R1000 |

* Number of reactions are based on 12 µL reaction volumes in Nanoplate 8.5k.

**Table 3. Wet-lab validated dPCR CNV Probe Centromeric Reference Assay (cat. no. 250213)**

| Detection Channel | Dye | Size* | GeneGlobe ID |
|---|---|---|---|
| Yellow | **HEX** | 300 rxns | DCC#######-H300 |
| Yellow | **HEX** | 1000 rxns | DCC#######-H1000 |
| Red | **ROX** | 300 rxns | DCC#######-R300 |
| Red | **ROX** | 1000 rxns | DCC#######-R1000 |

* Number of reactions are based on 12 µL reaction volumes in Nanoplate 8.5k.

# Custom dPCR CNV Probe Assays

**Table 4. Custom dPCR CNV Probe Assay (cat. no. 250214)**

| Detection Channel | Dye | Size* | GeneGlobe ID |
|---|---|---|---|
| Green | **FAM** | 250 rxns | CCP#######-F250 |
| Green | **FAM** | 600 rxns | CCP#######-F600 |
| Yellow | **HEX** | 250 rxns | CCP#######-H250 |
| Yellow | **HEX** | 600 rxns | CCP#######-H600 |
| Orange | ATTO 550 | 250 rxns | CCP#######-A250 |
| Orange | ATTO 550 | 600 rxns | CCP#######-A600 |
| Red | **ROX** | 250 rxns | CCP#######-R250 |
| Red | **ROX** | 600 rxns | CCP#######-R600 |
| Crimson | **Cy5** | 250 rxns | CCP#######-C250 |
| Crimson | **Cy5** | 600 rxns | CCP#######-C600 |

* Number of reactions are based on 12 µL reaction volumes in Nanoplate 8.5k.

# dPCR Instruments and Nanoplate Formats Compatible with dPCR CNV Probe Assays

**Table 5. dPCR Instruments and Nanoplate Formats Compatible with dPCR CNV Probe Assays**

| Type | Instruments | Instrument cat. nos. | Nanoplate formats | Nanoplate cat. nos. |
|---|---|---|---|---|
| 2 channels | QIAcuity One, 2plex | 911001 | 8.5k 24-well | 250011 |
| | | | 8.5k 96-well | 250021 |
| | | | 26k 8-well | 250031 |
| | | | 26k 24-well | 250001 |
| 5 channels | QIAcuity One, 5plex | 911021 | 8.5k 24-well | 250011 |
| | QIAcuity Four, 5plex | 911042 | 8.5k 96-well | 250021 |
| | QIAcuity Eight, 5plex | 911052 | 26k 8-well | 250031 |
| | | | 26k 24-well | 250001 |

# dPCR Mastermixes Compatible with dPCR CNV Probe Assays

**Table 6. dPCR Mastermixes Compatible with dPCR CNV Probe Assays**

| Name | Description | Mastermix cat. nos. |
|---|---|---|
| QIAcuity Probe PCR Kit | Standard Mastermix for probe-based assays in nanoplates on the QIAcuity | 250101, 250102, 250103 |
| QIAcuity UCP Probe PCR Kit | Ultra Clean Production Mastermix for probe-based assays in nanoplates on the QIAcuity | 250121, 250122 |
| QIAcuity One-Step Advanced Probe PCR Kit | One-step RT-PCR Mastermix optimized for the quantification of RNA and DNA targets in nanoplates on the QIAcuity | 250131, 250132 |

# Shipping and Storage

## Wet-lab Validated dPCR CNV Probe Assays

The wet-lab validated dPCR CNV Probe Assays (cat. no. 250210, 250212, and 250213) are shipped ready-to-use on dry ice. Upon receipt, the assays should be stored protected from light in a constant-temperature freezer at –30°C to –15°C for long-term storage (12 months) or at 2–8°C for short-term storage (6 months). Repeated freeze-thaw cycles should be avoided. If possible, store the assays in aliquots. Under these conditions, the components are stable, without showing any reduction in performance and quality.

## Custom dPCR CNV Probe Assays

The custom dPCR CNV Probe Assays (cat. no. 250214) are shipped lyophilized at ambient temperature. Upon receipt, the lyophilized custom dPCR CNV Probe Assays should be stored protected from light at 2–8°C for short term storage or at –30°C to –15°C in a constant-temperature freezer for long term storage. After re-suspension, it is recommended to store the CNV Assay at –30°C to –15°C. Repeated freeze-thaw cycles should be avoided. If possible, store the assay in aliquots. Under these conditions, the CNV Assay is stable until the expiry date listed on the vial.

# Intended Use

The dPCR CNV Probe Assays are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

# Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety**, where you can find, view, and print the SDS for each QIAGEN kit and kit component.

# Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of dPCR CNV Probe Assay is tested against predetermined specifications to ensure consistent product quality.

# Introduction

CNVs (copy number variations, or copy number alterations, CNAs) are structural changes in the genome (such as deletions, insertions, duplications, translocations, and inversions) that lead to gain or loss of copy numbers of a region, ranging from a few hundred base pairs up to whole chromosomes. CNVs are either inherited or the results of de novo structural changes. Responsible for up to 10–20% variation in the genome, CNVs are a source of natural genetic diversity as well as biological dysfunction in humans. CNVs often result in disruption of gene function, dosage imbalances, and positional effects, which are associated with complex diseases and traits such as cancer, obesity, or neurodegenerative and autoimmune diseases. The quantitative analysis of CNVs at disease-associated loci, therefore, can provide insights into the molecular mechanisms of diseases and potentially reveal novel biomarkers.

QIAGEN's dPCR CNV Probe Assays allow for highly sensitive and accurate detection of copy number changes for individual genes or regions of interest. QIAcuity CNV Probe dPCR assays can discriminate as little as 10% variation between samples, owing to the exceptional precision and resolution of copy number detection with QIAcuity dPCR technology. Other standard methods for CNV detection, such as microarrays, next-generation sequencing, and RT-PCR, lack the accuracy and sensitivity of copy number detection that is achievable by dPCR. These approaches fail to detect small changes in CNVs reliably, particularly at very low and very high DNA template concentrations.

# Principle and procedure

## Assay Design

All dPCR CNV Probe Assays are designed to detect unique regions in the human, mouse, or rat genome. The wet-lab validated dPCR CNV Probe Gene of Interest Assays target highly studied cancer and cancer-related genes in the human genome. If users wish to quantify gene targets for which no wet-lab validated assays exist, custom dPCR CNV Probe Assays can be designed and ordered at QIAGEN GeneGlobe.

The dPCR CNV Probe Reference Assays and dPCR CNV Probe Centromeric Reference Assays provide users with a choice of different reference targets in the human genome, making it possible to select the optimal normalization references for each analysis. Depending on the experimental conditions, multiple gene of interest (GOI) and reference gene assays can be included in the reactions for the accurate calculation of a gene of interest's copy number.

## Sample input amount

For optimal CNV analysis, expected copy numbers for target genes and reference genes should be taken into consideration when setting up reactions (if prior information is available). Total expected copies per reaction must lie within the dynamic detection range of the QIAcuity Digital PCR Nanoplate. Up to 170,000 copies per reaction can be detected when using the 8.5K Nanoplate, while up to 217,000 copies per reaction can be quantified with the QIAcuity 26K Nanoplate. This is based on the calculations shown in Table 7.

**Table 7. Nanoplates and their maximal copy numbers with hydrolysis probe chemistries**

| Nanoplate | Upper limit of copies per partition | Analyzed volume (µL) | Total reaction volume (µL) | Max copy number per analyzed volume | Estimated max copy number per reaction |
|---|---|---|---|---|---|
| 8.5k | 5 | Approx. 2.6 | 12 | 42,500 | 170,000 |
| 26k | 5 | Approx. 22.0 | 40 | 130,000 | 217,000 |

If the haploid genome size of the organism studied is known, the correlation between mass input of gDNA and the resulting copy number (for a single-copy gene) can be easily calculated, using the following formula:

Size of the haploid genome (bp) x average mass of a base pair ($1.096 \times 10^{-21}$ g/bp)

For the human genome with size of ca. $3.1 \times 10^9$ bp, the calculation is as follows:

$3.1 \times 10^9$ bp x $1.096 \times 10^{-21}$ g/bp = $3.3 \times 10^{-12}$ g = 3.4 pg per haploid genome

For human, mouse, and rat genomes, the copy number of single copy genes corresponding to different masses of genomic DNA of are presented in Table 8.

**Table 8. Conversion of DNA mass to copy number for human, mouse, and rat genomes**

| | | Approximate copy number of single-copy genes (1 copy per haploid genome) corresponding to the indicated amount of genomic DNA (gDNA) | | | |
|---|---|---|---|---|---|
| **Organism** | **Haploid Genome Size*** | **100 ng** | **10 ng** | **1 ng** | **0.1 ng** |
| Human (*Homo sapiens*) | $3.1 \times 10^9$ bp | 29,000 | 2900 | 290 | 29 |
| Mouse (*Mus musculus*) | $2.7 \times 10^9$ bp | 34,000 | 3400 | 340 | 34 |
| Rat (*Rattus norvegicus*) | $2.6 \times 10^9$ bp | 35,000 | 3500 | 350 | 35 |

* Genome sizes sourced from www.ensembl.org. Information is current as of November 2023. Genome size may change upon updates of the respective databases.

It is recommended to set up reactions with template input amounts resulting in 0.5–2 DNA copies/partition. This means:

- Approximately 17,000–100,000 copies per 12 µL 8.5k Nanoplate reaction (approximately 56–330 ng of human gDNA for a single-copy gene)
- Approximately 22,000–130,000 copies per 40 µL 26k Nanoplate reaction (approximately 73–430 ng of human gDNA for a single-copy gene).

However, the average number of copies per partition must not exceed 5. Furthermore, template amounts should not exceed 450 ng in a single dPCR reaction.

If the copy number input cannot be determined before starting the experiment, it is recommended to perform an initial titration experiment, using the unknown template in 2–4 tenfold dilutions to determine the optimal range for subsequent analyses.

## Restriction enzyme digestion

Fragmentation of DNA is particularly important when CNV analyses are performed, since multiple copies of a gene may be linked in tandem. Without fragmentation, linked copies of genes will be amplified in the same Nanoplate partition, resulting in an underquantification. Therefore, for the most accurate copy number determination, intact genomic DNA (e.g., samples extracted with the QIAamp® DNA Kit) should be fragmented via restriction enzyme digestion prior to quantification. For highly fragmented DNA (e.g., FFPE DNA or circulating DNA), restriction enzyme digestion is not need.

Restriction enzymes must not cut within the sequence of the PCR amplicon. During their design, each dPCR CNV Probe Assay has been checked for sequence overlap with 3 six-cutter restriction enzymes (EcoRI, PvuII, and XbaI) and 3 four-cutter restriction enzymes (AluI, CviQI, and HaeIII). Compatible restriction enzymes are indicated on the datasheet that is available for each assay.

For convenience, restriction digestion can be performed directly in the QIAcuity reaction mix. The restriction enzymes listed in the dPCR CNV Probe Assay datasheets have been validated to digest up to 700 ng of human genomic DNA in 10 min at room temperature in the QIAcuity Probe PCR Master Mix without impairing the subsequent PCR amplification. The restriction enzymes are simply added to mix during the reaction setup.

Alternatively, genomic DNA may be fragmented with restriction enzymes separately, before reaction setup. The fragmented template is then added to mix during reaction setup.

## Reference assay selection

Digital PCR allows for accurate estimation of copy number changes for target genes based on the ratio of absolute concentrations of target and reference genes within a sample. Including reference assays in dPCR tests serves other purposes as well, such as normalization of input templates due to pipetting errors or variation in the quality or quantity of DNA from sample to sample, especially when the origin of samples is unknown. Multiple reference assays can be quantitated simultaneously and independently, eliminating any sample variation or negative impact from potential pipetting errors.

The dPCR CNV Probe Assay portfolio offers multiple, wet-lab validated reference assays for human targets. Assays have been designed for the commonly used reference targets RPP30, TERT, APB3B, and AGO, which are present in 2 copies per diploid genome. It is important to note that the use of centromeric reference assays is advised when interrogating copy number variation in cancer cells or tumor biopsies. In cancer genomes, the structure of chromosome centromeres is more stable than that of the chromosome arms. For each autosome and for each sex chromosome, QIAGEN has developed a wet-lab validated centromeric reference assay.

**Table 9. List of wet-lab validated dPCR CNV Probe Reference Assays**

| Assay name | Copy number (diploid genome) | GeneGlobe® cat. no. |
|---|---|---|
| RPP30 | 2 | DCR0000181 |
| TERT | 2 | DCR0000186 |
| AP3B1 | 2 | DCR0000238 |
| AGO1 | 2 | DCR0000536 |
| Centromeric reference assays | 2 | Various |

## Multiplexing dPCR CNV Probe Assays with the QIAcuity Probe PCR Kit

The dPCR CNV Probe Assay portfolio is designed to work in conjunction with the QIAcuity Probe PCR Kit. The QIAcuity Probe PCR Kit contains a 4x concentrated, ready to use Master Mix optimized for microfluidic use in the QIAcuity Nanoplates. This special master mix enables accurate quantification of up to 5 targets having widely differing abundance within a single well of the QIAcuity Nanoplate. This saves time, money, and reduces the amount of sample material needed. Moreover, the duplex or multiplex PCR data obtained is comparable with that obtained from a singleplex PCR.

The QIAcuity Probe PCR Kit delivers singleplex or multiplex analysis with the highest specificity because of a novel, antibody-mediated, hot-start mechanism. At low temperatures, the QuantiNova® DNA Polymerase is kept in an inactive state by the QuantiNova Antibody and a novel additive, QuantiNova Guard, that stabilizes the complex. This improves the stringency of the hot-start and prevents extension of nonspecifically annealed primers and primer dimers. Within 2 minutes of raising the temperature to 95°C, the QuantiNova Antibody and QuantiNova Guard are denatured, and the QuantiNova DNA Polymerase is activated, enabling the PCR amplification.

The QIAcutiy Probe PCR Mastermix can be stored at 30°C for up to 100 hours without impairing the performance of subsequent reactions. The outstanding stability, even after

extended storage at room temperature without the use of any cooling agent, makes the QIAcuity Probe PCR Kit ideal for high-throughput reaction setup and plate-stack handling.

## Purification of DNA for determining copy number

The quantity and purity of a DNA sample can affect the performance of the QIAcuity dPCR Assays for CNV analysis. It is highly recommended to refer to the following guidelines prior to setting up dPCR reactions.

### DNA purification

When starting with purification of genomic DNA, an appropriate purification method should be used depending on the starting material and desired amount of purified genomic DNA. The kits listed below are recommended for genomic DNA purification from various types of starting material for use with QIAGEN's dPCR Copy Number Assays (Table 10). DNA purification can be carried out according to the kit instructions.

**Table 10. Recommended DNA purification kits for use with QIAcuity dPCR CNV Probe Assays**

| Starting material | Purification method | Cat. no. |
|---|---|---|
| Fresh or frozen tissues, cultured cells | QIAamp DNA Mini Kits | 51304 and 51306 |
| | DNeasy® Blood & Tissue Kits | 69506, 69504, 69582, and 69581 |
| | EZ1& EZ2™ DNA Investigator Kit | 952034 |
| | EZ1&2 DNA Tissue Kit | 953034 |
| | Blood & Cell Culture DNA Mini, Midi, or Maxi Kit | 13323, 13343, or 13362, respectively |
| | FlexiGene® DNA Kit | 51206 |
| | PAXgene® Tissue DNA Kit | 767134 |
| FFPE tissues | QIAamp® DNA FFPE Tissue Kit | 56404 |
| | QIAamp DNA FFPE Advanced UNG Kit | 56704 |
| cfDNA from human plasma or serum | QIAamp DNA Blood Mini or Maxi Kits | 51106, or 51194 and 51192, respectively |
| | PAXgene Blood DNA Kit | 761133 |
| | QIAamp ccfDNA/RNA Kit | 55184 |
| | EZ1&2 ccfDNA Kit | 954854 |
| | QIAamp MinElute ccfDNA Midi and Mini Kits | 55284 and 55204, respectively |
| Other biological samples | Contact QIAGEN Technical Service | |

**Important**: Do not use diethyl pyrocarbonate (DEPC)–treated water.

**Important**: Presence of RNA in the sample preparations will result in inaccurate DNA concentration measurements. Removing RNA contamination with an RNase A digestion is highly recommended.

**Important**: Including no template control samples (NTCs) in the experimental design will provide information about presence of any DNA contaminants.

## DNA quality

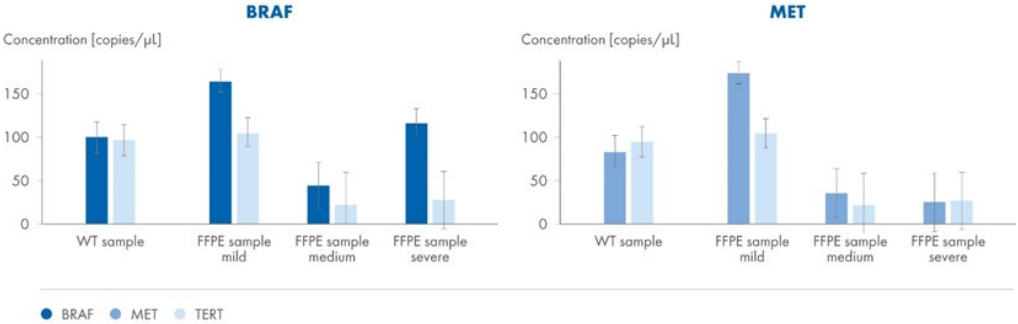
All DNA samples used in reaction mixes should show similar quality and quantity, which can easily be assessed using ultraviolet (UV) spectrophotometry.

DNA samples measured with a UV spectrophotometer should meet the following criteria:

- Concentration determined by $A_{260}$ should be >10 µg/mL.
- $A_{260}/A_{230}$ ratio should be greater than 1.7.
- $A_{260}/A_{280}$ ratio should be greater than 1.8.

Because water is not buffered, the pH and the resulting $A_{260}/A_{280}$ ratio can vary greatly. Lower pH results in a lower $A_{260}/A_{280}$ ratio and reduced sensitivity to protein contamination. For accurate values, we recommend measuring absorbance in 10 mm Tris·Cl, pH 7.5. For DNA samples are that diluted in 10mM Tris Cl, pH 8.0, an absorbance reading of 1.0 at 260 nm in a 1 cm detection path corresponds to a DNA concentration of 50 ug/µL.

Quality-compromised input material, such as DNA samples extracted from FFPE samples with varying degrees of crosslinking and fragmentation, may result in suboptimal CNV detection (Figure 1) due to possible fragmentation of the genomic region that harbors the gene of interest or target of interest.




| | CN/genome | |
|---|---|---|
| **Sample** | **BRAF** | **MET** |
| WT | 2 | 2 |
| FFPE mild | 2.99 | 3.82 |
| FFPE medium | 3.79 | 3.69 |
| FFPE severe | 8.24 | 2.07 |

**Figure 1. Use of DNA templates with compromised quality (here, fragmented FFPE material) might result in inaccurate CNV detection.** Examples given here are target assays BRAF and MET, which were tested using genomic DNA extracted from FFPE tissue material with varying levels of fragmentation and formalin damage (mild, medium, and severe). With increasing levels of fixation and fragmentation, copy number per genome of both BRAF and MET target assays changed drastically. These results indicate that copy number determination is inaccurate and inconsistent in "severely" fragmented and formalin-compromised FFPE material, when compared to FFPE material with "mild" and "medium" levels of fragmentation and fixation.

# Equipment and Reagents to be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- Genomic DNA isolation kit (refer to Table 10, page 16, for DNA purification kit recommendations)
- QIAcuity Digital PCR Instrument (refer to Table 5, page 5, for the appropriate dPCR cyclers)
- QIAcuity dPCR Nanoplates (refer to Table 5, page 5, for the appropriate nanoplates)
- QIAcuity Probe PCR Mastermix
- Multichannel pipettor
- Nuclease-free pipette tips and tubes

# Important Notes

For accurate and reproducible dPCR results, it is essential to avoid contamination of the assay with foreign DNA, especially PCR products from previously run nanoplates. The most common sources of DNA contamination are the products of previous experiments and highly concentrated template dilutions.

To maintain a working environment free of DNA contamination, we recommend the following precautions:

- Wear gloves throughout the procedure. Use only fresh PCR-grade labware (tips and tubes).
- Use sterile pipette tips with filters.
- Store and extract positive materials (specimens, positive controls, and amplicons) separately from all other reagents.
- Physically separate the workspaces used for dPCR setup and post-dPCR processing operations. Decontaminate your dPCR workspace and labware (pipette, tube racks, etc.) with UV light before each new use to render any contaminated DNA ineffective in dPCR through the formation of thymidine dimers or with 10% bleach to chemically inactivate and degrade any DNA.
- Do not open any previously run and stored dPCR nanoplate. Removing the sealer foil from dPCR nanoplate releases dPCR product DNA into the air where it can contaminate the results of future experiments. In the event of contamination ensure that any affected labware and bench surfaces are decontaminated.
- Do not remove the dPCR nanoplate from its protective sealed bag until immediately before use.

# Protocol: dPCR CNV Probe Assays

This protocol is optimized for the quantification of gene-specific regions in human, mouse, or rat DNA using the dPCR CNV Probe Assays (cat. nos. 250210, 250212, 250213, 250214) with the QIAcuity® Probe PCR Kit (cat. nos. 250101, 250102, 250103) in a singleplex or multiplex reaction using the QIAcuity digital PCR (dPCR) instrument, to detect copy number variations (CNV). The dPCR CNV Probe Assays are available in 5 different dyes: FAM, HEX, Atto550, ROX, and Cy5. For further information, please refer to the dPCR CNV Probe Assays product page at **www.qiagen.com/dPCRCNVProbeAssays**

## Important points before starting

- A reference target assay with a known copy number per genome can be used to identify CNVs for target(s) of interest in test samples. For consistency of CNV analysis, the same reference target assay(s) and target-of-interest assay(s) should be used across test samples.
- We recommend using multiple reference target assays depending on experimental conditions. Refer to the *QIAcuity User Manual Extension: QIAcuity Application Guide* for more information on reference target assay selection.
- For best results, all DNA samples should be resuspended in DNase-free water or, alternatively, in DNase-free 10 mM Tris buffer, pH 8.0.
- The quality and source of any additional positive and negative control sample(s) be comparable to that of test samples, if possible.
- dPCR CNV Probe Assays are used as a 20x primer/probe mix. Always start with the 20x assay concentration and dPCR cycling conditions in this protocol.
- Pipetting accuracy and precision affects the consistency of results. Be sure that all pipettes and instruments have been checked and calibrated according to the manufacturer's recommendations. Also make sure that no bubbles are introduced into the wells of the QIAcuity Nanoplate during pipetting.

## Things to do before starting

- Custom dPCR CNV Probe Assays must be reconstituted with Nuclease-free water to a 20x concentration according to their product data sheets, which are available for download in the user's GeneGlobe® account.
- Determine DNA concentration and purity by preparing dilutions and measuring absorbance in 10 mM Tris, pH 8.0 buffer. For best results, the concentration measured at $A_{260}$ should be greater than 10 ng/µL DNA, the $A_{260}/A_{280}$ ratio should be greater than 1.8.
- Thaw genomic DNA and QIAcuity Probe Mastermix on ice (4°C). After thawing, mix gently by repeated pipetting or quick vortex, then quick spin.

## Template DNA Digestion

- Before partitioning, DNA samples with an average length ≥20 kb should be digested. This ensures accurate and precise quantification. DNA fragmentation via restriction digestion is particularly important when CNV analyses are performed where multiple copies of a gene might be linked in tandem. Restriction digestion is not required for highly fragmented DNA (e.g., FFPE DNA or circulating DNA).
- The restriction enzymes in Table 11 are validated to digest template DNA in 10 min at RT in QIAcuity Probe PCR Master Mix without impairing the subsequent PCR amplification. For assay-specific restriction enzyme compatibility, please go to **geneglobe.qiagen.com** or refer to the product data sheet that is provided with each assay.

**Table 11. List of recommended enzymes**

| 6-Cutter restriction enzymes | | 4-Cutter restriction enzymes | |
|---|---|---|---|
| EcoRI | 0.25 U/µL EcoRI-HF®, NEB*<br>0.025 U/µL Anza™ 11 EcoRI, TFS† | AluI | 0.025 U/µL AluI, NEB*<br>0.025 U/µL Anza 44 AluI, TFS |
| PvuII | 0.025 U/µL PvuII, NEB<br>0.025 U/µL Anza 52 PvuII, TFS | CviQI | 0.025 U/µL CviQI, NEB<br>0.025 U/µL Csp6I (CviQI), TFS |
| XbaI | 0.025 U/µL Anza 12 XbaI, TFS | HaeII | 0.025 U/µL BsuRI (HaeIII), TFS |

* NEB, New England Biolabs.
† TFS, Thermo Fisher Scientific.

# Procedure

## Reaction setup

1. Thaw the QIAcuity Probe PCR Master Mix, template DNA, dPCR CNV Probe Assays, and RNase-free water. Mix the individual solutions.
2. Prepare a reaction mix according to Table 12. Due to the hot-start, it is not necessary to keep samples on ice during reaction setup or while programming the QIAcuity instrument.
3. Vortex the reaction mix.

**Table 12. Reaction setup**

| | Volume/reaction | | |
|---|---|---|---|
| **Component** | **Recommended: Nanoplate 8.5k (24-well, 96-well)** | **Optional: Nanoplate 26k (8-well, 24-well)** | **Final concentration** |
| 4x Probe PCR Master Mix | 3 µL | 10 µL | 1x |
| 20x dPCR CNV Probe Assay 1* | 0.6 µL | 2 µL | 1x |
| 20x dPCR CNV Probe Assay 2,3,4,5* | 0.6 µL | 2 µL | 1x |
| Restriction Enzyme† (highly recommended‡) | 0.3–3 units | 1–10 units | 0.025–0.25 U/µL |
| RNase-free water | Variable | Variable | |
| Template gDNA (added at step 4) | Variable§ | Variable§ | |
| **Total reaction volume** | **12 µL** | **40 µL** | |

* For respective dye recommendations for the CNV Probe Assays and available channels on QIAcuity, as well as for multiplexing, please see the *QIAcuity User Manual* or the *QIAcuity User Manual Extension: QIAcuity Application Guide*.

† For selection of restriction enzymes, please refer to assay specifications and the *QIAcuity User Manual Extension: QIAcuity Application Guide*.

‡ For gDNA with an average length ≥20 kB .

§ Template gDNA amounts ideally should lie within 30–100 ng/reaction and should not exceed 450 ng/reaction when detecting CNV targets present in 2 copies/diploid genome.

4. Dispense appropriate volumes of the reaction mix, which contains all components except the template, into the wells of a standard PCR plate. Then, add template DNA into each well that contains the reaction mix.

**Note**: The appropriate amounts of reaction mix and template DNA depends on various parameters. Please refer to the *QIAcuity User Manual Extension: QIAcuity Application Guide* for details.

5. Transfer the contents of each well of the standard PCR plate to the wells of a fresh nanoplate.
6. Seal the nanoplate properly using the QIAcuity Nanoplate Seal provided in the QIAcuity Nanoplate Kits. For exact sealing procedure, see the *QIAcuity User Manual*.
7. If a restriction enzyme for DNA digestion has been included in the reaction, leave the plate for 10 min at room temperature (15–25°C).

## Thermal cycling and imaging conditions

1. Set the cycling conditions under the dPCR parameters in the QIAcuity Software Suite or at the QIAcuity instrument according to Table 13.

**Table 13. Cycling conditions**

| Step | Time | Temperature |
|---|---|---|
| **PCR initial heat activation** | 2 min | 95°C |
| **2-step cycling (40 cycles)** | | |
| Denaturation | 15 s | 95°C |
| Combined Annealing/Extension | 30 s | 60°C |

2. Activate the required channels in **Imaging**, under the dPCR parameters in the QIAcuity Software Suite or at the QIAcuity instrument. The imaging settings should be set to the values outlined in Table 14.

**Table 14. Imaging settings conditions**

| Channel | Exposure duration (ms) | Gain |
|---|---|---|
| **Green** | 500 | 6 |
| **Yellow** | 500 | 6 |
| Orange | 400 | 6 |
| **Red** | 300 | 4 |
| **Crimson** | 400 | 8 |

3. Place the nanoplate into the QIAcuity instrument and start the dPCR program.

## Data analysis

4. To set up a plate layout according to the experimental design, open the QIAcuity Software Suite and define the reaction mixes, samples, and controls. The plate layout can be defined before or after the nanoplate run.

   **Note**: Refer to the *QIAcuity User Manual* for details on setting up the plate layout.

5. After the nanoplate run, the raw data is automatically sent to the QIAcuity Software suite.
6. For data analysis, open the QIAcuity Software Suite and select the individual nanoplate for the analysis in **Plate Overview** of the software suite.

   **Note**: Refer to the *QIAcuity User Manual Extension: QIAcuity Application Guide* or the *QIAcuity User Manual* for details on how to analyze the data and calculate copy numbers per human genome based on the reference gene assay(s) you have added to the nanoplate.

# Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: **www.qiagen.com/FAQ/FAQList.aspx**. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, visit **www.qiagen.com**).

| | | Comments and suggestions |
|---|---|---|
| **Weak or no signal** | | |
| a) | The selected fluorescence channel for dPCR data analysis does not comply with the protocol | For data analysis, select one of the 5 channels, green, yellow, orange, red, or crimson depending on the probe dye used. |
| b) | Incorrect programming of the QIAcuity dPCR instrument | Compare the temperature profile with the protocol. See the cycling conditions in Table 13. Refer to the *QIAcuity User Manual*. |
| c) | PCR extension time too short | Use the extension time specified in the protocol. |
| d) | PCR was inhibited | Use the recommended DNA isolation method and closely follow the manufacturer's instructions. QIAGEN offers dedicated sample preparation kits developed to complement dPCR CNV Probe Assays, and to provide a complete and efficient workflow for CNV determination. |
| e) | Incorrect configuration of the PCR | Ensure that reactions were set up according to the reaction mix preparation described in Table 12. Repeat the dPCR run if necessary. |
| f) | The storage conditions for one or more kit components did not comply with the instructions given in "Shipping and Storage". | Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary. |
| g) | The dPCR CNV Probe Assay has expired | Check the storage conditions and the expiration date of the reagents and use a new kit, if necessary. |
| h) | Insufficient starting template | Increase the amount of template genomic DNA. |
| i) | Restriction enzyme cuts within amplicon | If restriction enzymes cut within the PCR amplicon sequence, little or no signal will be generated. Confirm that the correct restriction enzyme |

| | | Comments and suggestions |
|---|---|---|
| | | was chosen for the experiment and repeat. Alternatively, use another restriction enzyme to perform DNA digestion. |
| **Signals present for the negative control template (NTC copies/µL > 0)** | | |
| a) | Contamination occurred during PCR setup | Repeat the PCR with new reagents.<br>If possible, seal the PCR array/close the PCR tubes directly after addition of the sample to be tested.<br>Make sure to pipette the positive controls last.<br>Make sure that workspace and instruments are decontaminated at regular intervals. |
| b) | Contamination occurred during extraction | Repeat the extraction and PCR of the sample to be tested using new reagents.<br>Make sure that workspace and instruments are decontaminated at regular intervals. |
| c) | Custom dPCR CNV Probe Assay Design is sub-optimal | Custom dPCR CNV Probe Assays are not wet-lab validated. If possible, choose another assay design for the target of interest and repeat the experiment. |
| **Underquantification of target** | | |
| a) | Incorrect programming of the QIAcuity dPCR instrument | Compare the temperature profile with the protocol. See the cycling conditions in Table 13. Refer to the *QIAcuity User Manual*. |
| b) | PCR extension time too short | Use the extension time specified in the protocol in Table 13. |
| c) | PCR was inhibited | Use the recommended DNA isolation method and closely follow the manufacturer's instructions. QIAGEN offers dedicated sample preparation kits developed to complement dPCR CNV Probe Assays, and to provide a complete and efficient workflow for CNV determination. |
| d) | Incorrect configuration of the PCR | Ensure that reactions were set up according to the reaction mix preparation described in Table 12. Repeat the dPCR run, if necessary. |
| e) | Suboptimal choice of reference gene | The choice of reference assay can potentially result in an artificial relative underquantification of targets of interest. If possible, use multiple reference gene assays to ensure that normalization is not impacted by reference assay bias. Furthermore, when investigating cancer samples, be sure to use centromeric reference assays. The instability of cancer genomes can result in copy number variations even in commonly used reference genes located on the chromosome arm. Centromeric regions are more stable than chromosome arms, making them a better choice for this application. |

| | | Comments and suggestions |
|---|---|---|
| f) | Tandemly linked gene copies segregated into the same partition | Without fragmentation, linked copies of genes will be amplified in the same Nanoplate partition, resulting in an underquantification. Intact genomic DNA must be fragmented via restriction enzyme digestion prior to quantification. If restriction enzyme digestion was performed, try digestion with another restriction enzyme. To increase the likelihood that linked genes will be separated from one another, use a compatible 4-cutter restriction enzyme to fragment the sample DNA, since it will cut the genome more frequently than a 6-cutter restriction enzyme. |
| g) | Structure of locus containing PCR targets is challenging for PCR reaction | For some loci in the genome, the structure of the DNA can prove challenging for PCR reactions. Without proper fragmentation, challenging DNA structures can lead to the underquantification of DNA targets contained within them. If restriction enzyme digestion was performed, try digestion with another restriction enzyme. If possible, use a compatible 4-cutter restriction enzyme to fragment the sample DNA, since it will cut the genome more frequently than a 6-cutter restriction enzyme. |
| h) | Custom dPCR CNV Probe Assay Design is sub-optimal | Custom dPCR CNV Probe Assays are not wet-lab validated. If possible, choose another assay design for the target of interest and repeat the experiment. |
| i) | Incorrect programming of the QIAcuity dPCR instrument | Compare the temperature profile with the protocol. See the cycling conditions in Table 13. Refer to the *QIAcuity User Manual*. |
| **Overquantification of target** | | |
| a) | Suboptimal choice of reference gene | The choice of reference assay can potentially result in an artificial relative overquantification of targets of interest. If possible, use multiple reference gene assays to ensure that normalization is not impacted by reference assay bias. Furthermore, when investigating cancer samples, be sure to use centromeric reference assays. The instability of cancer genomes can result in copy number variations even in commonly used reference genes located on the chromosome arm. Centromeric regions are more stable than chromosome arms, making them a better choice for this application. |
| b) | Custom dPCR CNV Probe Assay Design is sub-optimal | Custom dPCR CNV Probe Assays are not wet-lab validated. If possible, choose another assay design for the target of interest and repeat the experiment. |
| **Suboptimal 1D Scatterplots (e.g. rain, low fluorescence, fluorescence saturation)** | | |
| a) | Incorrect programming of the QIAcuity dPCR instrument | Compare the temperature profile with the protocol. See the cycling conditions in Table 13. Refer to the *QIAcuity User Manual*. |
| b) | PCR extension time too short | Use the extension time specified in the protocol. |

| | | Comments and suggestions |
|---|---|---|
| c) | PCR was inhibited | Use the recommended DNA isolation method and closely follow the manufacturer's instructions. QIAGEN offers dedicated sample preparation kits developed to complement dPCR CNV Probe Assays, and to provide a complete and efficient workflow for CNV determination. |
| d) | Incorrect configuration of the PCR | Ensure that reactions were set up according to the reaction mix preparation described in Table 12. Repeat the dPCR run, if necessary. |
| e) | The storage conditions for one or more kit components did not comply with the instructions given in "Shipping and Storage". | Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary. |
| f) | Structure of locus containing PCR targets is challenging for PCR reaction | For some loci in the genome, the structure of the DNA can prove challenging for PCR reactions. Without proper fragmentation, challenging DNA structures can lead to rainy 1D scatterplots or positive amplification clusters with low fluorescence. If restriction enzyme digestion was performed, try digestion with another restriction enzyme. If possible, use a compatible 4-cutter restriction enzyme to fragment the sample DNA, since it will cut the genome more frequently than a 6-cutter restriction enzyme. |
| g) | Custom dPCR CNV Probe Assay Design is sub-optimal | Custom dPCR CNV Probe Assays are not wet-lab validated. If possible, choose another assay design for the target of interest and repeat the experiment. |
| h) | Custom dPCR CNV Probe Assay was reconstituted incorrectly (too dilute) | If Custom dPCR CNV Probe Assays are reconstituted incorrectly, such that they are too dilute, the end fluorescence in the 1D Scatterplots will be weaker than normal. If customers know by which factor the assay was incorrectly reconstituted, they can compensate for this error during reaction setup by adding the appropriate amount more of the assay.<br>If the assay was diluted such that it no longer fits into the reaction setup, customers may evaporate the assay in a vacuum concentrator (e.g. SpeedVac™). Following evaporation, the assay may be reconstituted again using the correct volume of nuclease-free water indicated in its accompanying product data sheet. |
| i) | Custom dPCR CNV Probe Assay was reconstituted incorrectly (too concentrated) | If Custom dPCR CNV Probe Assays are reconstituted incorrectly, such that they are too concentrated, the end fluorescence in the 1D Scatterplots will be much stronger than normal, potentially reaching saturation. If customers know by which factor the assay was incorrectly reconstituted, they can compensate for this error during reaction setup by adding less of the assay to their reaction mixes. |

# Contact Information

For technical assistance and more information, please see our Technical Support Center at **www.qiagen.com/Support**, call 00800-22-44-6000, or contact one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit **www.qiagen.com**).

# Appendix A: Custom dPCR CNV Probe Asssays Design at GeneGlobe

Users have the option to design custom dPCR CNV Probe Assays for gene targets at **geneglobe.qiagen.com**. Each custom dPCR CNV Probe Assay can be designed for detection in the Green, Yellow, Orange, Red, or Crimson channel of the QIAcuity.

## Input information for human, mouse, and rat targets

For human, mouse, and rat targets, any of the following input information can be used to define targets for assay design:

- Gene symbol
- Chromosome coordinates
- Pasted DNA sequence (up to 250 nt in length)

When designing assays for targets in a given organism (e.g. human ATF3), assay designs will be checked for cross-reactivity with the rest of the respective genome (e.g., human genome).

## Input information for organisms other than human, mouse, or rat

For all other organisms, custom designs can be made using:

- Pasted DNA sequence (up to 250 nt in length)

For targets in other organisms, the assays will be design principles will be the same as those used for human, mouse, and rat targets. However, assay designs will be not checked for cross-reactivity with any genome.

## Output information for custom designs

For each defined target, up to five assay designs will be generated. Each assay design will be accompanied by the following information

- Design quality score (excellent, good, poor)
- Visual display of assay location in genome browser
- Amplicon size
- Restriction enzyme(s) with which an assay is compatible. Each assay is checked for compatibility with EcoRI, PvuII, XbaI, AluI, CviQI, and XbaI.

## Ordering and Delivery

Following assay design, users can order one or all of the assay designs. At this point, users will need to define:

- the scale of the assay order (e.g., the number of 12 µL 8.5K Nanoplate reactions possible)
- the desired detection channel (e.g., which fluorophore will be used for the hydrolysis probe)

Each ordered assay is delivered lyophilized. A product data sheet containing detailed information pertaining to the assay is available in the user's GeneGlobe account. This includes instructions for reconstituting the lyophilized assay.

## Reordering

To reorder assays, customers can use the custom GeneGlobe ID (GGID) provided with the assay.

# Ordering Information

| Product | Contents | Cat. no. |
|---|---|---|
| dPCR CNV Probe Gene of Interest Assay (variable) | 20x concentrated, ready to use wet-lab validated dPCR CNV Probe Gene of Interest Assay | 250210 |
| dPCR CNV Probe Reference Assay (variable) | 20x concentrated, ready to use wet-lab validated dPCR CNV Probe Reference Assay | 250212 |
| dPCR CNV Probe Centromeric Reference Assay (variable) | 20x concentrated, ready to use wet-lab validated dPCR CNV Probe Centromeric Reference Assay | 250213 |
| Custom dPCR CNV Probe Assay | Lyophilized custom dPCR CNV Probe Assay | 250214 |
| **Accessories** | | |
| QIAcuity UCP Probe PCR Kit (5ml) | 5 tubes of 1.00 mL 4x concentrated QIAcuity UCP Probe Mastermix each, 8 tubes of 1.9 mL UCP water each. | 250122 |
| Nanoplate 24-well 26K | Microfluidic digital PCR plates for 24 samples with up to 26,000 partitions each | 250001, 250002 |
| Nanoplate 24-well 8.5K | Microfluidic digital PCR plates for 24 samples with up to 8,500 partitions each | 250011 |
| Nanoplate 96-well 8.5K | Microfluidic digital PCR plates for 96 samples with up to 8,500 partitions each | 250021 |
| Nanoplate Seals | 11 top seals for the nanoplates | 250099 |

| Product | Contents | Cat. no. |
|---|---|---|
| QIAcuity One, 2plex | One-plate digital PCR instrument for detecting up to 2 fluorescent dyes | 911001 |
| QIAcuity One, 5plex | One-plate digital PCR instrument for detecting up to 5 fluorescent dyes | 911021 |
| QIAcuity Four, 5plex | Four-plate digital PCR instrument for detecting up to 5 fluorescent dyes | 911042 |
| QIAcuity Eight, 5plex | Eight-plate digital PCR instrument for detecting up to 5 fluorescent dyes | 911052 |
| QIAcuity UCP Probe PCR Kit (5mL) | 5 tubes of 1mL 4x concentrated QIAcuity UCP Probe Mastermix each, 8 tubes of 1.9 mL UCP water each. | 250122 |
| Nanoplate 24-well 26k | Microfluidic digital PCR plates for 24 samples with up to 26,000 partitions each | 250001, 250002 |
| Nanoplate 24-well 8.5k | Microfluidic digital PCR plates for 24 samples with up to 8500 partitions each | 250011 |
| Nanoplate 96-well 8.5k | Microfluidic digital PCR plates for 96 samples with up to 8500 partitions each | 250021 |
| Nanoplate Seals | 11 top seals for the nanoplates | 250099 |
| **Related Products** | | |
| QIAcuity One-Step Viral RT-PCR Kit | 4 x 1.3 mL One-Step Viral RT-PCR Master Mix (4x), 2x 100 µL Multiplex Reverse Transcription Mix (100x) and 8x 1.9 mL RNase-Free Water; for 500 reactions in Nanoplate 26k and 1666 reactions in Nanoplate 8.5k | 1123145 |

| Product | Contents | Cat. no. |
|---|---|---|
| QIAcuity One-Step Advanced Probe PCR Kit (1 mL) | 1 mL OneStep Advanced Probe Master Mix (4x), 45 µL OneStep RT Mix (100x), 1 mL Enhancer GC, 20 µL QN Internal Control RNA, 2 x 1.9 mL RNase-free water; for 100 reactions in Nanoplate 26K and 333 reactions in Nanoplate 8.5K | 250131 |
| QIAcuity One-Step Advanced Probe PCR Kit (5 mL) | 5 x 1 mL OneStep Advanced Probe Master Mix (4x), 5 x 45 µL OneStep RT Mix (100x), 5 x 1 mL Enhancer GC, 1 x 20 µL QN Internal Control RNA, 8 x 1.9 mL RNase-free water; for 500 reactions in Nanoplate 26K and 1666 reactions in Nanoplate 8.5K | 250132 |
| QIAamp DNA Mini Kit (50) | For 50 DNA preps: 50 QIAamp Mini Spin Columns, QIAGEN Proteinase K, Reagents, Buffers, Collection Tubes (2 mL) | 51304 |
| QIAamp DNA Mini Kit (250) | For 250 DNA preps: 250 QIAamp Mini Spin Columns, QIAGEN Proteinase K, Reagents, Buffers, Collection Tubes (2 mL) | 51306 |
| DNeasy Blood & Tissue Kit (250) | 250 DNeasy Mini Spin Columns, Proteinase K, Buffers, Collection Tubes (2 mL) | 69506 |
| DNeasy Blood & Tissue Kit (50) | 50 DNeasy Mini Spin Columns, Proteinase K, Buffers, Collection Tubes (2 mL) | 69504 |

| Product | Contents | Cat. no. |
|---|---|---|
| DNeasy 96 Blood & Tissue Kit (12) | For 12 x 96 DNA minipreps: 12 DNeasy 96 Plates, Proteinase K, Buffers, S-Blocks, AirPore Tape Sheets, Collection Microtubes (1.2 mL), Elution Microtubes RS, Caps, 96-Well Plate Registers | 69582 |
| QIAcuity One-Step Advanced Probe PCR Kit (1 ml) | 1 mL OneStep Advanced Probe Master Mix (4x), 45 µL OneStep RT Mix (100x), 1 mL Enhancer GC, 20 µL QN Internal Control RNA, 2 x 1.9 mL RNase-free water; for 100 reactions in Nanoplate 26K and 333 reactions in Nanoplate 8.5K | 250131 |
| DNeasy 96 Blood & Tissue Kit (4) | For 4 x 96 DNA minipreps: 4 DNeasy 96 Plates, Proteinase K, Buffers, S-Blocks, AirPore Tape Sheets, Collection Microtubes (1.2 mL), Elution Microtubes RS, Caps, 96-Well Plate Registers | 69581 |
| EZ1&2 DNA Investigator Kit (48) | For 48 preps: Reagent Cartridges (DNA Investigator), Disposable Filter-Tips, Disposa-ble Tip-Holders, Sample Tubes (2 mL), Elution Tubes (1.5 mL), Buffer G2, Proteinase K, Carrier RNA | 952034 |
| EZ1&2 DNA Tissue Kit (48) | For 48 preps: Reagent Cartridges (Tissue), Disposable Filter-Tips, Disposable Tip-Holders, Sample Tubes (2 mL), Elution Tubes (1.5 mL), Buffer G2, Proteinase K, Carrier RNA | 953034 |
| Blood & Cell Culture DNA Mini Kit (25) | 25 QIAGEN Genomic-tip 20/G, QIAGEN Protease, Buffers | 13323 |

| Product | Contents | Cat. no. |
|---|---|---|
| Blood & Cell Culture DNA Midi Kit (25) | 25 QIAGEN Genomic-tip 100/G, QIAGEN Protease, Buffers | 13343 |
| Blood & Cell Culture DNA Maxi Kit (10) | 10 QIAGEN Genomic-tip 500/G, QIAGEN Protease, Buffers | 13362 |
| FlexiGene DNA Kit (250) | For isolation of genomic DNA from whole blood, buffy coat, and cultured cells in a single tube with fast kit preparation | 51206 |
| PAXgene Tissue DNA Kit (50) | For 50 DNA preps: PAXgene DNA Mini Spin Columns, Processing Tubes, Microcentrifuge Tubes, Carrier RNA, and Buffers; to be used in conjunction with PAXgene Tissue Containers | 767134 |
| QIAamp DNA FFPE Tissue Kit (50) | For 50 DNA preps: 50 QIAamp MinElute Columns, Proteinase K, Buffers, Collection Tubes (2 mL) | 56404 |
| QIAamp DNA FFPE Advanced UNG Kit (50) | For 50 preps: Uracil-N-glycosylase, QIAamp UCP MinElute columns, collection tubes, Deparaffinization Solution, Proteinase K, RNase A, RNase-free water and buffers | 56704 |
| QIAamp DNA Blood Mini Kit (250) | For 250 DNA minipreps: 250 QIAamp Mini Spin Columns, QIAGEN Protease, Reagents, Buffers, Collection Tubes (2 mL) | 51106 |
| QIAamp DNA Blood Maxi Kit (50) | For 50 DNA maxipreps: 50 QIAamp Maxi Spin Columns, QIAGEN Protease, Buffers, Collection Tubes (50 mL) | 51194 |

| Product | Contents | Cat. no. |
|---|---|---|
| QIAamp DNA Blood Maxi Kit (10) | For 10 DNA maxipreps: 10 QIAamp Maxi Spin Columns, QIAGEN Protease, Buffers, Collection Tubes (50 mL) | 51192 |
| PAXgene Blood DNA Kit (25) | Processing tubes and buffers for 25 preparations | 761133 |
| QIAamp ccfDNA/RNA Kit (50) | For 50 preps: RNeasy Midi and RNeasy MinElute Spin Columns, Collection Tubes (50 mL), Elution Tubes (1.5 mL and 2 mL), RNase-Free Reagents and Buffers | 55184 |
| EZ1&2 ccfDNA Kit (48) | For 48 preps (2, 4, or 8 mL sample input volume each): 48 reagent cartridges (EZ1&2 ccfDNA), Magnetic Bead Suspension, Elution Buffer, Large-Volume Tubes (7 mL), Disposable Tip Holders, Disposable Filter-Tips, Elution Tubes (1.5 mL) | 954854 |
| QIAamp MinElute ccfDNA Midi Kit (50) | For 50 preps (4 or 5 mL sample input volume each): QIAamp UCP MinElute Columns, QIAGEN Proteinase K, Magnetic Bead Suspension, Buffers, Bead Elution Tubes, and Collection Tubes (1.5 mL and 2 mL) | 55284 |
| QIAamp MinElute ccfDNA Mini Kit (50) | For 50 preps (1 or 2 mL sample input volume each): QIAamp UCP MinElute Columns, QIAGEN Proteinase K, Magnetic Bead Suspension, Buffers, Bead Elution Tubes, Collection Tubes (1.5 mL and 2 mL) | 55204 |

| Product | Contents | Cat. no. |
|---|---|---|
| **QIAcube Connect — for fully automated nucleic acid extraction with QIAGEN spin-column kits** | | |
| QIAcube Connect* | Instrument, connectivity package, 1-year warranty on parts and labor | Inquire |

* All QIAcube Connect instruments are provided with a region-specific connectivity package, including tablet and equipment necessary to connect to the local network. Further, QIAGEN offers comprehensive instrument service products, including service agreements, installation, introductory training and preventive subscription. Contact your local sales representative to learn about your options.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

# Document Revision History

| Revision | Description |
|---|---|
| 03/2024 | Initial release. |