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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

Table of Contents

- Kit Contents 3
 - Wet-lab tested dPCR CNV Probe Assays 3
 - Custom dPCR CNV Probe Assays 4
 - dPCR Instruments and Nanoplate Formats Compatible with dPCR CNV Probe Assays ... 5
 - dPCR Mastermixes Compatible with dPCR CNV Probe Assays 5
- Shipping and Storage 6
- Intended Use 7
- Safety Information 8
- Quality Control 8
- Introduction 9
 - Principle and procedure 10
 - Purification of DNA for determining copy number 15
- Equipment and Reagents to be Supplied by User 19
- Important Notes 20
- Protocol: dPCR CNV Probe Assays 21
 - Important points before starting 21
 - Things to do before starting 22
 - Template DNA Digestion 22
 - Procedure 23
- Troubleshooting Guide 29
- Contact Information 34
- Appendix A: Custom dPCR CNV Probe Assays Design at GeneGlobe 35
- Ordering Information 37
- Document Revision History 42

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 tested. If users wish to quantify gene targets for which no wet-lab tested assays are available, custom dPCR CNV Probe Assays for targets in human, mouse, and rat can be designed and ordered at QIAGEN GeneGlobe.

Wet-lab tested dPCR CNV Probe Assays

Table 1. Wet-lab tested 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
Far Red	ATTO 700	500 rxns	DCG#####S500

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

Table 2. Wet-lab tested 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 tested 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
Far Red	ATTO 700	250 rxns	CCP#####S250
Far Red	ATTO 700	600 rxns	CCP#####S600

* 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
QIAcuity High Multiplex Probe PCR Kit	Allows multiplexing of up to 12 assays in one reaction on QIAcuity instruments with QIAcuity Software Suite version 3.1 or higher	250133, 250134

Shipping and Storage

Wet-lab tested dPCR CNV Probe Assays

The wet-lab tested 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 $2-8^{\circ}\text{C}$ 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 $2-8^{\circ}\text{C}$ 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 tested 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:

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

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

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

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)	3.1×10^9 bp	29,000	2900	290	29
Mouse (Mus musculus)	2.7×10^9 bp	34,000	3400	340	34
Rat (Rattus norvegicus)	2.6×10^9 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 needed.

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.

Important: To ensure complete DNA digestion, it is essential that the specific versions of the restriction enzymes from the indicated suppliers are used.

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 tested 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 QIAcuity 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.

Multiplexing dPCR CNV Probe Assays with the QIAcuity High Multiplex Probe PCR Kit

The dPCR CNV Probe Assay portfolio and the Custom dPCR CNV Probe Assays also work with the QIAcuity High Multiplex Probe PCR Kit. With an optimized buffer and the highest concentration of Taq polymerase in any QIAcuity Master Mix, this mix can be used to quantify up to 12 DNA targets per reaction; thereby reducing the time, money, and amount of sample

needed. For more details on higher order multiplexing, refer to the *QIAcuity High Multiplex Probe PCR Kit handbook*.

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

Table 10. Recommended DNA purification kits for use with QIAcuity dPCR CNV Probe Assays (continued)

Starting material	Purification method	Cat. no.
	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 $>10\text{ }\mu\text{g/mL}$
- 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 that are diluted in 10 mM 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 $\mu\text{g}/\mu\text{L}$.

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.

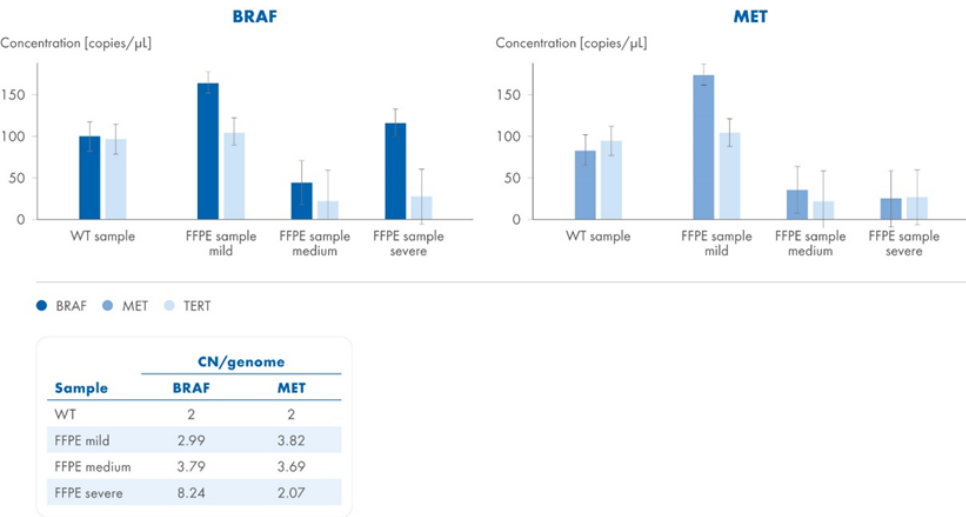


Figure 1. Use of DNA templates with compromised quality (here, fragmented FFPE material) might result in inaccurate CNV detection. Examples given here are target assays BRAF and MET, which were tested using genomic DNA extracted from FFPE tissue material with varying levels of fragmentation and formalin 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 for DNA purification kit recommendations)
- QIAcuity Digital PCR Instrument (refer to Table 5 for the appropriate dPCR cyclers)
- QIAcuity dPCR Nanoplates (refer to Table 5 for the appropriate nanoplates)
- QIAcuity Probe PCR Kit
- QIAcuity High Multiplex Probe PCR Kit
- 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, and 250214) with the QIAcuity® Probe PCR Kit (cat. nos. 250101, 250102, and 250103) or QIAcuity High Multiplex Probe PCR Kit (cat. nos. 250133 and 250134) 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 6 different dyes: FAM, HEX, ATTO 550, ROX, Cy5, and ATTO 700. For further information, please refer to the dPCR CNV Probe Assays product page at www.qiagen.com

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.
- 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 affect the consistency of results. Ensure that no bubbles are introduced into Nanoplate wells while pipetting.
- Pipetting accuracy and precision affects the consistency of results. Be sure that all pipets 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.

- The QIAcuity High Multiplex Probe PCR Kit requires the QIAcuity Software Suite version 3.0 or later.

Things to do before starting

- Reconstitute custom dPCR CNV Probe Assays to a 20x concentration with nuclease-free water according to their product datasheets, which can be retrieved from each user's GeneGlobe account.
- Thaw genomic DNA and QIAcuity Probe Master Mix or QIAcuity High Multiplex Probe PCR Master Mix 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 of ≥ 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 and QIAcuity High Multiplex 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.
- **Important:** To ensure complete DNA digestion, it is essential that the specific versions of the restriction enzymes from the indicated suppliers are used.

Table 11. List of recommended enzymes

6-Cutter restriction enzymes		4-Cutter restriction enzymes	
EcoRI	0.25 U/μL EcoRI-HF®, NEB * 0.025 U/μL Anza™ 11 EcoRI, TFS†	AluI	0.025 U/μL AluI, NEB 0.025 U/μL Anza 44 AluI, TFS
PvuII	0.025 U/μL PvuII, NEB 0.025 U/μL Anza 52 PvuII, TFS	CviQI	0.025 U/μL CviQI, NEB 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 selected 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, Table 13, or Table 14 (excluding the DNA template), depending on the master mix and application. 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 for QIAcuity Probe PCR Master Mix

Component	Volume/reaction		Final concentration
	Recommended: Nanoplate 8.5k (24-well, 96-well)	Optional: Nanoplate 26k (8-well, 24-well)	
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 (each)	2 µL (each)	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.

Table 13. Reaction setup for QIAcuity High Multiplex Probe PCR Master Mix for detecting up to 6 targets per reaction (one target per channel in up to 6 channels)

Component	Volume/reaction		Final concentration
	Nanoplate 8.5k (24-well, 96-well)	Nanoplate 26k (8-well, 24-well)	
4x QIAcuity High Multiplex Probe PCR Master Mix	3 µL	10 µL	1x
20x Primer–probe mix 1–6*	0.6 µL (each)	2 µL (each)	1x
Restriction Enzyme† (optional‡)	Up to 1 µL	Up to 1 µL	0.025–0.25 U/µL
RNase-free water	Variable	Variable	–
Template DNA (added at step 4)	Variable§	Variable§	–
Total reaction volume	12 µL	40 µL	–

* For respective dye recommendations, refer to 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.

Table 14. Reaction setup for QIAcuity High Multiplex Probe PCR Master Mix for detecting up to 12 targets per reaction with amplitude-based multiplexing

Component	Volume/reaction		Final concentration
	Nanoplate 8.5k (24-well, 96-well)	Nanoplate 26k (8-well, 24-well)	
4x QIAcuity High Multiplex Probe PCR Master Mix	3 µL	10 µL	1x
20x Primer–probe mix 1–12* (for multiplex)	Variable	Variable	Variable†
Restriction Enzyme‡ (optional§)	Up to 1 µL	Up to 1 µL	0.025–0.25 U/µL
RNase-free water	Variable	Variable	–
Template DNA (added at step 4)	Variable¶	Variable¶	–
Total reaction volume	12 µL	40 µL	–

* For respective dye recommendations, refer to the *QIAcuity User Manual* or the *QIAcuity User Manual Extension: QIAcuity Application Guide*.

† For reactions that employ amplitude-based multiplexing, variable assay concentrations are required. Refer to the *QIAcuity High Multiplex Probe PCR Kit Handbook* for more details.

‡ 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 26k or 8.5k 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 Cycling conditions.
2. Activate the required channels in Imaging, under the dPCR parameters in the QIAcuity Software Suite or at the QIAcuity instrument. Imaging settings should be set to the default values.

Note: If using the QIAcuity High Multiplex Probe PCR Kit, the “Enable High-Multiplexing-Reference channel” toggle in the Imaging tab must be activated. Otherwise, NO valid wells will appear during analysis. Refer to the *QIAcuity High Multiplex Probe PCR Kit Handbook* for recommendations on the maximum RFU levels for each channel when performing higher-order multiplexing.

Table 15. Cycling conditions

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

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

Data analysis

1. 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.

2. After the nanoplate run, the raw data is automatically sent to the QIAcuity Software Suite.
3. 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* and the *QIAcuity User Manual* for details on how to analyze the data and calculate copy numbers per human genome based on the reference(s) you have added to the nanoplate.

Note: The “Copy Number Variation” analysis option in the QIAcuity Software Suite is not available when amplitude-based multiplexing is used.

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 15. 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, Table 13, or Table 14. 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.

Comments and suggestions

- | | |
|--|---|
| 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 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.
If possible, seal the PCR array/close the PCR tubes directly after addition of the sample to be tested.
Make sure to pipette the positive controls last.
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.
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 15. Refer to the QIAcuity User Manual. |
| b. PCR extension time too short | Use the extension time specified in the protocol in . |
| 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, Table 13, or Table 14. Repeat the dPCR run, if necessary. |

Comments and suggestions

- | | |
|--|---|
| 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. |
| 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 15. 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. |
|--|--|

Comments and suggestions

- | | |
|--|--|
| 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 . Refer to the QIAcuity User Manual. |
| b. PCR extension time too short | Use the extension time specified in the protocol. |
| 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, Table 13, or Table 14. 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. |

Comments and suggestions

- | | |
|---|--|
| h. Custom dPCR CNV Probe Assay was reconstituted incorrectly (too dilute) | <p>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.</p> <p>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.</p> |
| i. Custom dPCR CNV Probe Assay was reconstituted incorrectly (too concentrated) | <p>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.</p> |

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 Assays 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 assay 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 μ L 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
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 1 mL 4x concentrated QIAcuity UCP Probe Mastermix each, 8 tubes of 1.9 mL UCP water each.	250122

Product	Contents	Cat. no.
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
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
QIAcuity High Multiplex Probe PCR Kit (1 mL)	1 mL 4x concentrated QIAcuity HighMultiplex Probe PCR Mastermix, 2x 1.9mL Rnase-free Water, QN Internal Control DNA dPCR	250133
QIAcuity High Multiplex Probe PCR Kit (5mL)	5 x 1mL 4x concentrated QIAcuity HighMultiplex Probe PCR Mastermix, 8 x 1.9mL Rnase-free Water, QN Internal Control DNA dPCR	250134
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

Product	Contents	Cat. no.
DNeasy Blood & Tissue Kit (50)	50 DNeasy Mini Spin Columns, Proteinase K, Buffers, Collection Tubes (2 mL)	69504
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, Disposable 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
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

Product	Contents	Cat. no.
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
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
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.
02/2025	Inclusion of QIAcuity High Multiplex Probe PCR Kits (cat. nos. 250133 and 250134).

Limited License Agreement for dPCR Copy Number Variation (CNV) Probe Assays

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