March 2024

Quick-Start Protocol

dPCR PanCancer Kit

This protocol is optimized for the detection of multiple mutations in the specified target gene using the dPCR PanCancer Kits for BRAF V600 mutations and EGFR exon19 deletions (cat. no. 250284, 250287) using QIAGEN®'s QIAcuity® instruments for digital PCR (dPCR). Each kit consists of 2 boxes, one with the PanCancer Assay reagents and one with the QIAcuity MasterMix and water

The dPCR PanCancer Kit reagents should be stored immediately upon receipt at –30°C to –15°C in a constant-temperature freezer and protected from light. Unless otherwise indicated on the label, the components are stable until the expiration date indicated on the kit without showing any reduction in performance under these conditions.

Dedicated protocols for the various types of QIAGEN's QIAcuity dPCR assays can be found in their respective quick-start protocols and the *QIAcuity User Manual Extension: Application Guide*.

Further information

- *QIAcuity User Manual Extension: Application Guide*: **www.qiagen.com/HB-2839**
- *QIAcuity User Manual*: **www.qiagen.com/HB-2717**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Set up all reaction mixtures in an area separate from that used for DNA isolation and PCR product analysis (post-PCR).
- Use disposable tips containing hydrophobic filters to minimize cross-contamination.
- Always use the cycling conditions specified in the protocol. The cycling conditions have been optimized for this assay.
- Dilutions of DNA quantification standards in QuantiTect® Nucleic Acid Dilution Buffer can be stored at 4°C for at least 1 week.
- **Important:** The 2nd level analysis tool for mutation frequencies within the QIAcuity software suite cannot be used for the PanCancer assay results because it employs a distinct calculation metric specific for the LNA Mutation assays (cat. no. 250200). See "Calculation of mutation frequencies" section in the *dPCR PanCancer Kit handbook* (**www.qiagen.com/HB-3542**) for further details.

Template DNA digestion

DNA samples with ≥30 kb average length (e.g., genomic DNA purified via spin column with silica membrane, or salting out method) should be fragmented by restriction digestion before partitioning. Enzymatic fragmentation of larger DNA ensures even distribution of template throughout the partitions of the QIAcuity Nanoplate well, which in turn leads to accurate and precise quantification.

Restriction digestion is not required for highly fragmented DNA, e.g., FFPE DNA or circulating cell free DNA (ccfDNA).

Care should be taken to use enzymes that will not cut within the amplified sequence. For QIAGEN's PanCancer Assays digestion with the 4-cutter BsuRI (HaeIII) from Thermo Fisher Scientific is recommended. This validated enzyme will digest DNA in 10 min at room temperature (15–25°C) when added directly to the QIAcuity reaction mix at a concentrations

of 0.025 U/µL. To ensure complete DNA digestion, it is essential that the specific versions of the restriction enzymes from the indicated suppliers are used.

Procedure: Reaction Setup

Thaw the QIAcuity MasterMix, template DNA, PanCancer Assay, reference assay and RNase-free water. Vigorously mix the QIAcuity MasterMix and the individual solutions. Centrifuge briefly to collect liquids at the bottom of the tubes.

1. Prepare a reaction mix for the number of reactions needed according to Table 1. Due to the hot-start capability, it is not necessary to keep samples on ice during reaction setup or while programming the QIAcuity instrument.

Table 1. Reaction setup

	Volume/reaction	
Component	**Nanoplate 26k (24-well)**	**Final concentration**
4x QIAcuity MasterMix	10 µL	1x
20x PanCancer Assay	2 µL	1x
20x Reference Assay AP3B1	2 µL	1x
Restriction Enzyme (optional)	Up to 1 µL	0.025–0.25 U/µl
RNAse-free water	Variable	
Template DNA (added at step 5)	Variable*	
Total reaction volume	**40 µL**	

* Appropriate template amount depends on various parameters. Please see the *QIAcuity User Manual Extension: Application Guide* for details.

Note: Prepare a volume of master mix 10% greater than that required for the total number of PCR assays to be performed. This should include positive and negative control reactions.

2. Mix the master mix thoroughly.
3. Dispense appropriate volumes of the reaction mix, which contains all components except the template into the wells of a standard PCR plate.

4. Then add template DNA into each well that contains the reaction mix and mix thoroughly.
5. Transfer the content of each well from the standard PCR plate into the wells of the nanoplate.
6. Seal the nanoplate properly using the QIAcuity Nanoplate Seal provided in the QIAcuity Nanoplate Kits.
7. If a restriction enzyme for DNA digestion has been included in the reaction, leave the plate for 10 min at room temperature.
8. Program the cycler of the QIAcuity instrument according to Table 2.

Table 2. Thermal cycling conditions

Step	Time	Temperature (°C)
PCR initial heat activation	2 min	95
2-step cycling (35 cycles*)		
Denaturation	15 s	95
Combined annealing/extension	20 s	55

* Number of cycles might vary depending on sample type. Additional 5 cycles might increase signal to noise separation.

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

Document Revision History

Date	Changes
03/2024	Initial release

Scan QR code for the *QIAcuity User Manual Extension: Application Guide*.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.