# The use of a dPCR LNA® Mutation Assay (QIAGEN® GeneGlobe) on the QIAcuityDx® System in Utility (open) Mode


## Abstract

The QIAcuityDx System is a digital PCR (dPCR) platform that incorporates in vitro diagnostic (IVD) accessories, including a Universal MasterMix for dPCR (QIAcuityDx Universal MasterMix Kit) that includes a separate vial of $MgCl_2$+ to aid with optimization efforts, as well as a 26k partition, 24-well nanoplate (QIAcuityDx Nanoplate 26k 24-well). The integrated software also includes a Utility Mode that supports custom assay development by offering suitable flexibility for the assay setup and reporting options. The QIAcuityDx Four in Utility Mode and QIAcuityDx Universal MasterMix have been specifically designed to support using the QIAcuityDx System for non-IVD digital PCR assays, and enable the development of user-specific workflows that have the best performance.

Our dPCR LNA Mutation Assays comprise a range of dPCR assays that use locked nucleic acids (LNA) and have been tested in the wet lab. These assays are for research purposes, cover more than 200 targets related to cancer and oncogenesis and are available from QIAGEN GeneGlobe. While dPCR LNA Mutation Assays provide good results under the recommended conditions in most situations, in rare cases assay conditions might require some minor adjustment to best meet the requirements of the user's workflow.


## Introduction

Our oncology related dPCR LNA Mutation Assays are designed for detecting specific DNA sequence mutations related to cancer and oncogenesis, and are meant for non-clinical research purposes. The assays are duplex in nature and designed to detect mutated and wild type sequences in a choice of FAM® + HEX® or Atto 550 + ROX® fluorescent dye combinations (Figure 1A). Our dPCR LNA Mutation Assays are suitable to use with circulating tumor DNA, liquid biopsy, FFPE and other tissue samples and can be used with QIAcuityDx Universal MasterMix Kits or QIAcuity® Probe PCR Kits. The LNA-enhanced primers and probes help increase assay specificity and sensitivity.


**dPCR LNA Mutation Assays**

## Considerations

The QIAcuityDx integrated software includes a Utility Mode that supports custom assay development by offering suitable flexibility for the assay setup and reporting options. In this mode the dPCR parameters such as plate configuration and cycling parameters can be easily adjusted to suit the user's required assay conditions.

Prior to running an assay for the first time on the QIAcuityDx Four instrument in the Utility Mode of the software, we recommend the selected assay(s) be tested to ensure the best performance in the user setting and workflow (1). While dPCR LNA Mutation Assays provide sensitive and specific mutation detection using the recommended conditions in the majority of situations (Figure 1B, EGFR L858R), some assays might require minor adjustment to meet the requirements of the user workflow and sample type. This is particularly the case if challenging sample types are used. Samples regarded as challenging include those containing degraded or fragmented DNA or difficult background matrices, such as patient samples or those containing inhibitors. We highly recommend using QIAGEN extraction technologies to minimize these issues.

## Test setup

Testing should encompass the use of mutation-positive (Mut) and wild type (WT) samples to demonstrate the specificity of the assay to the target mutation in the user workflow, as illustrated in Figure 1B. Samples of the appropriate type (e.g., genomic DNA (gDNA)) should be included to examine any effect the sample background matrix may have on the assay. Once established, such samples can be used as positive controls for subsequent experiments. Controls without a template should also be used to confirm the assay and MasterMix reagents have not become contaminated by environmental DNA.

## Testing sensitivity

Sensitivity should also be examined by testing titrations of the Mut sample, preferably in a background of the appropriate sample type (Figure 2). This information can help to define the sensitivity and working range limits of the assays in the user workflow.

## Adjusting conditions

Adjustments to the standard starting setup conditions and instrument settings recommended for the assays and QIAcuityDx Four instrument (available in the QIAcuityDx User Manual) might be required to maximize the assay performance (Figure 3) (2, 3). Most commonly, the threshold might need to be adjusted to separate the positive and negative partitions correctly. Testing with known Mut and WT samples can guide these adjustments. As illustrated in Figure 3, the threshold might need to be adjusted upwards to avoid artifacts such as crosstalk from a strong positive sample in another color channel or non-specific amplification. These effects might show as partitions with an elevated fluorescence compared with the baseline, yet not as high as true positive partitions in a positive sample.

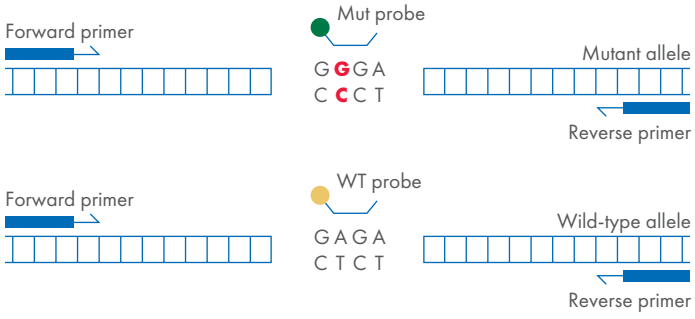


**Figure 1A**
Our dPCR LNA Mutation Assays are duplex assays designed to detect mutated and wild type sequences in a choice of FAM + HEX or Atto 550 + ROX fluorescent dye combinations.

Adjusting the imaging parameters, exposure time and gain might also be necessary for best performance. Details of imaging adjustment can be found in the QIAcuityDx User Manual Section 5.15 (3). The imaging conditions should be adjusted to yield fluorescence values for positive partitions in the range of 80–120 RFU. The fluorescence of negative partitions should be as low as possible for the best separation of positive and negative partitions, and therefore an accurate threshold setting.

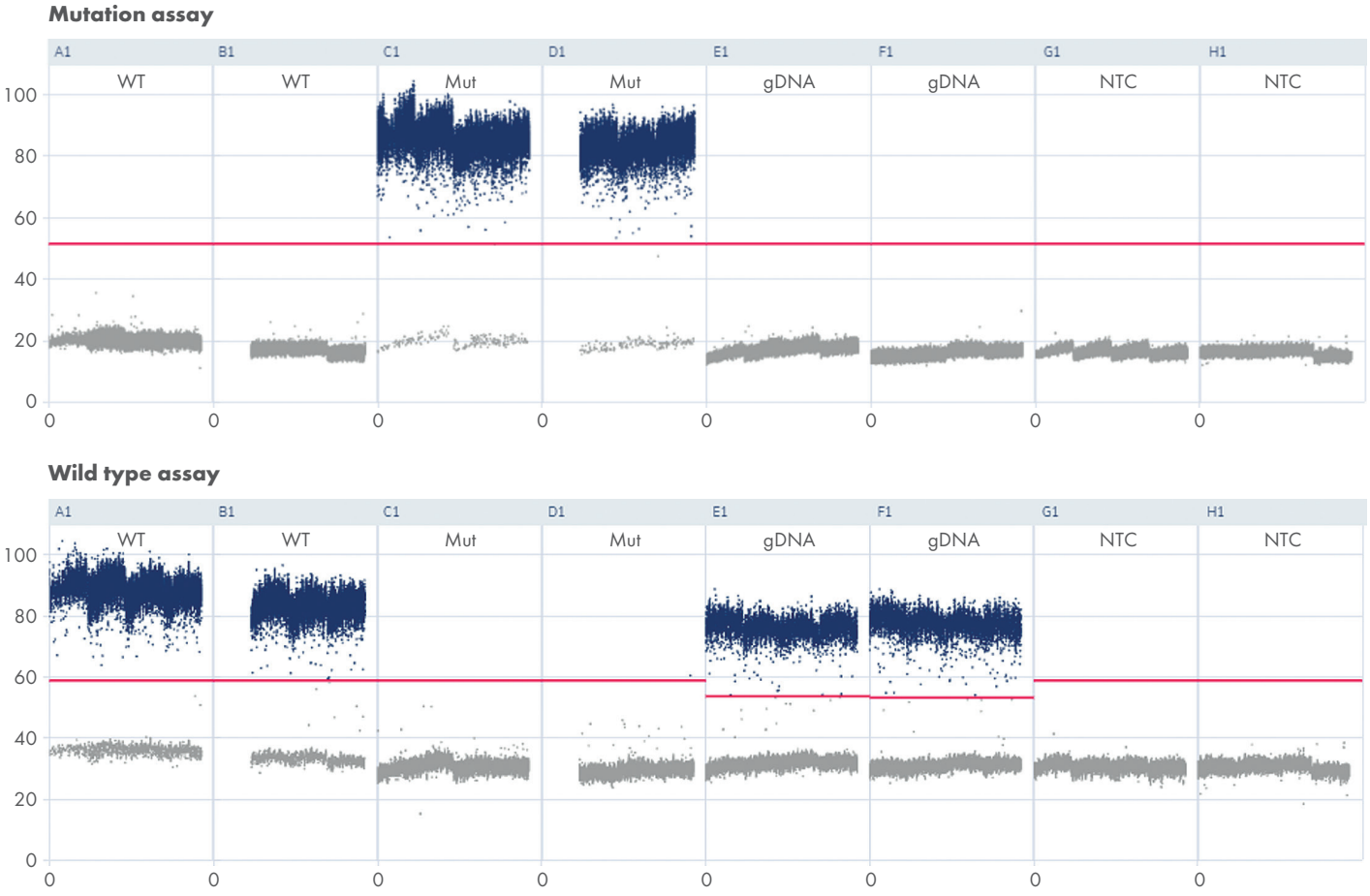


**Figure 1B**

EGFR L858R mutation assay (c.2573T>G; GeneGlobe ID - DMH0000386) tested with Mut and WT templates, comprising long oligonucleotides that bear the appropriate sequences (IDT, Lueven, Belgium). The mutation assay, shown above in the FAM channel, and wild type assay, shown in the lower row in the HEX channel, give the expected result from the respective templates at default conditions on the QIAcuityDx platform in Utility Mode. Positive and negative partitioned fluorescence are well separated, allowing thresholds to be set easily using either automated or manual adjustment options. To further confirm the specificity of the assay, appropriate sample types should be tested. In this case gDNA was used, and indicates correct amplification of the wild type assay. NTC: No template control.

## Adjusting assay conditions

When running any new assay for dPCR (or for qPCR), the first step is usually to demonstrate sensitive and specific detection of mutations. Yet in some cases, such as those with challenging sample types as previously mentioned, adjustment to the recommended starting conditions might be required to achieve the desired performance. For instance, if a high percentage of partitions are distributed across a range of fluorescence between positive and negative partition clusters ("rain"), or if non-specific amplification makes threshold setting difficult (Figure 3).

In this circumstance we recommend the primer and probe concentration, magnesium chloride concentration and the thermal profile conditions (in particular, the annealing temperature and time) be optimized for best results. Details are available in the QIAcuityDx Universal Mastermix Kit Handbook (4).

QIAcuityDx Universal MasterMix Kit is designed to give sensitive and specific results from most assays using the recommended default conditions. If optimization is required, however, the QIAcuityDx Universal MasterMix Kit includes magnesium chloride as a separate component to allow adjustment, with a recommended starting range of 2–6 mM (4).

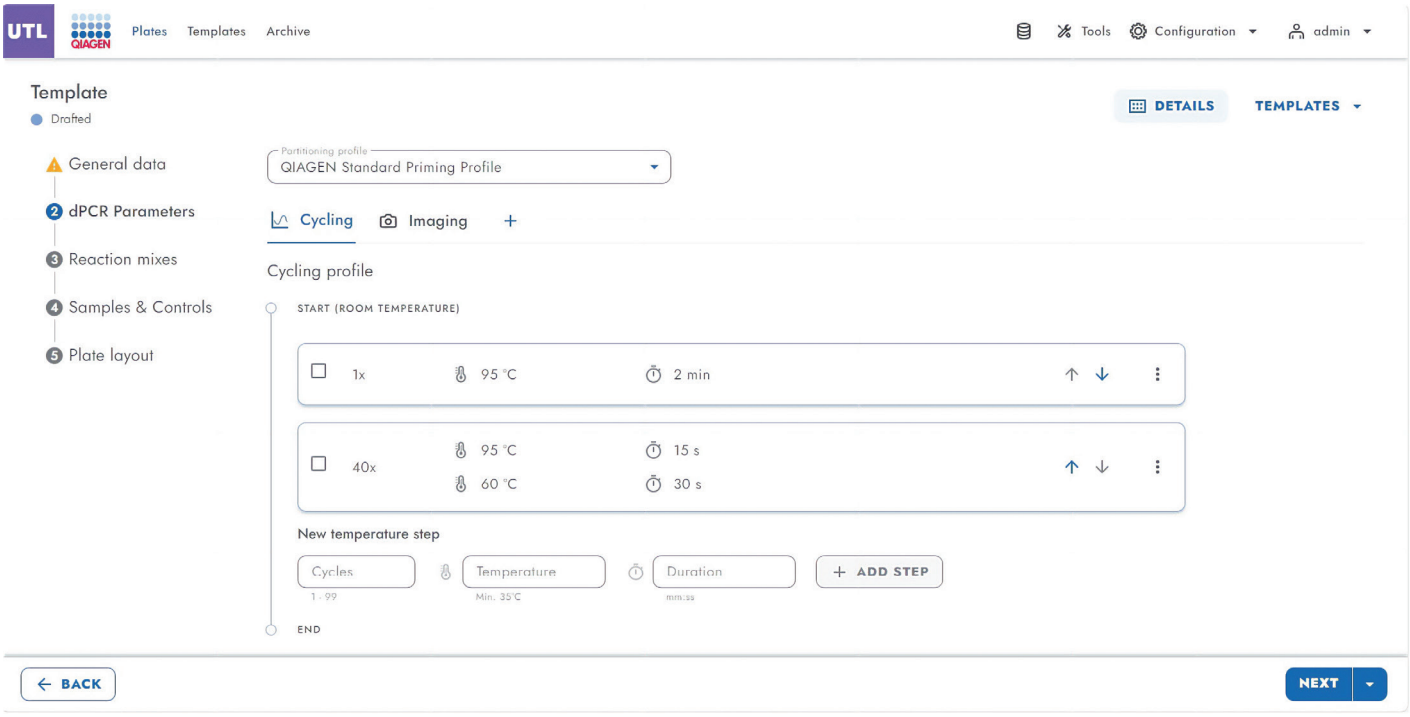


**Figure 1C**
The recommended initial cycling conditions for our dPCR LNA Mutation Assays. While the standard conditions illustrated should yield sensitive and specific mutation detection in most cases, adjustments may be required in some workflows. Typically this would involve changes to the annealing temperature or time (60°C for 30-second step). Further details are available in the QIAcuityDx Universal MasterMix Kit Handbook (4).

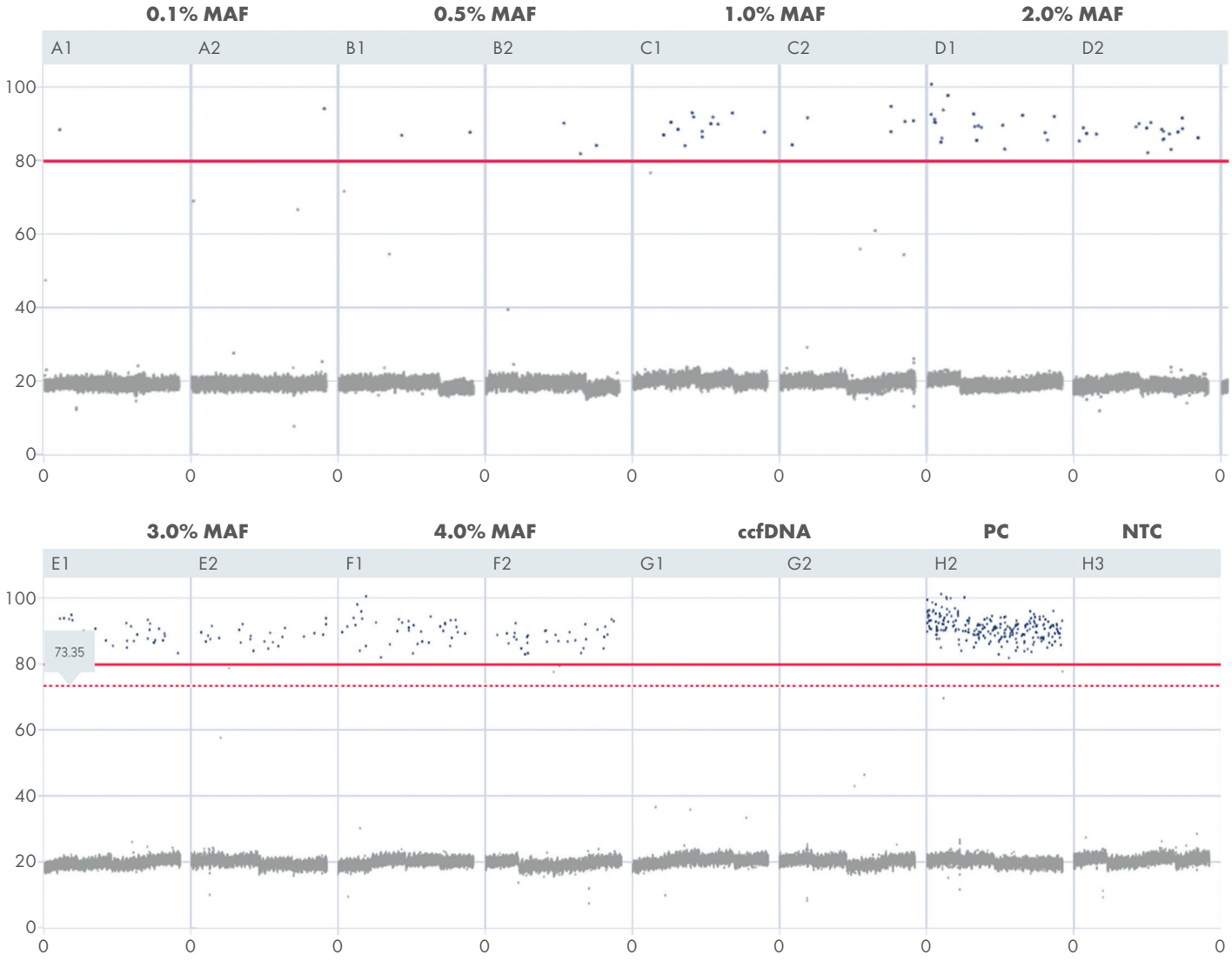


**Figure 2**

EGFR L858R mutation assay tested using a Mut template. The template is comprised of an oligonucleotide spiked into circulating cell-free DNA (ccfDNA) which was extracted from healthy donor plasma using QIAamp® DSP Circulating NA Kit. Sensitivity should be examined by testing titrations of a Mut sample, preferably in a background of the appropriate sample type. Here mutant detection can be observed to 0.1% mutant allele frequency (MAF) in a ccfDNA background. PC: Positive control; NTC: No template control.

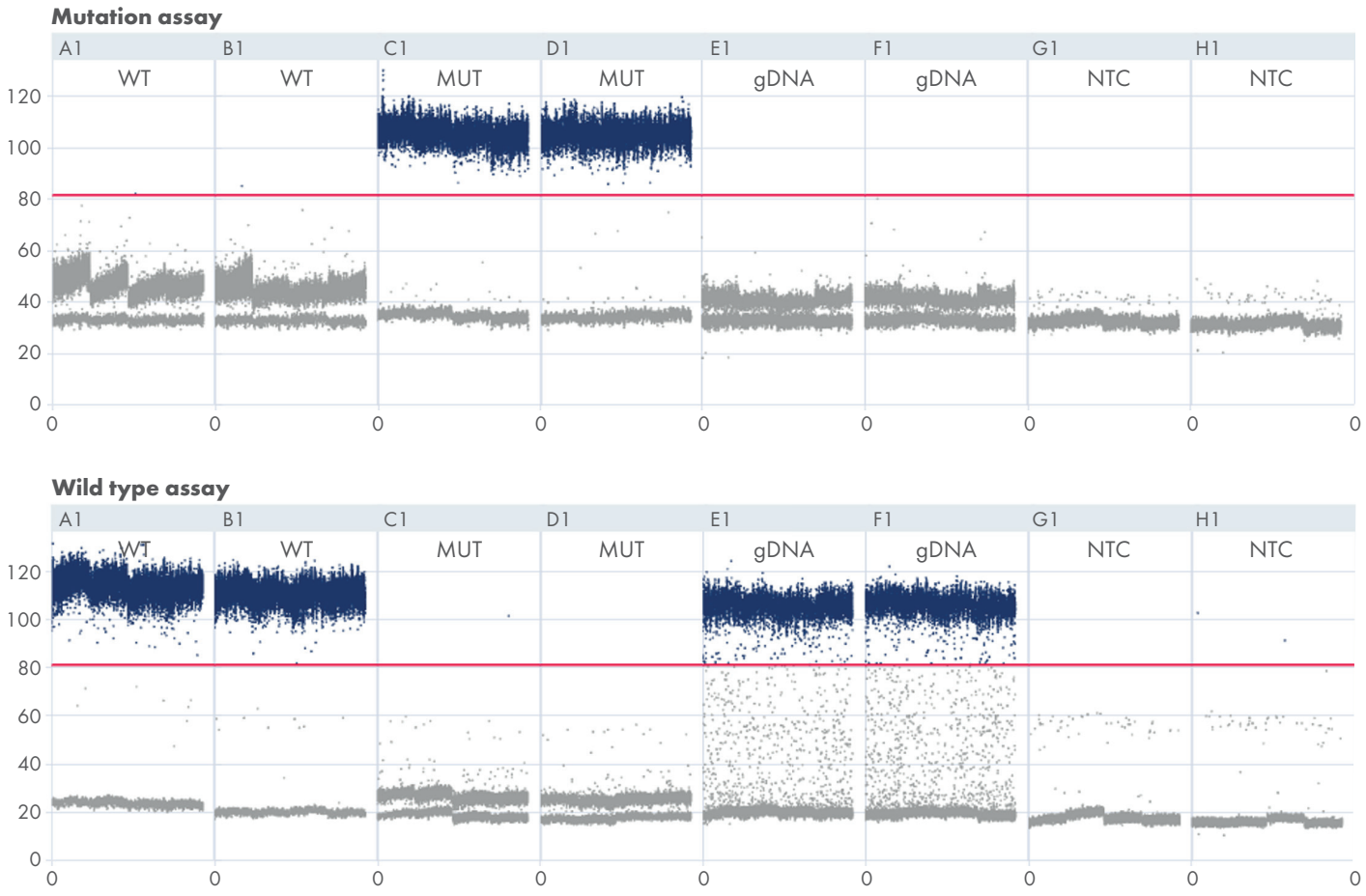


**Figure 3**

KRAS G12C assay tested on the QIAcuityDx Four in Utility Mode. The mutation assay is in the green channel (top row) and the wild type assay is in the yellow channel (bottom row). Mut (well C1, D1) and WT (well A1, B1) oligonucleotide templates can inform threshold setting in the presence of cross talk artifacts (green channel A1, B1; yellow channel C1, D1) and non-specific amplification (E1-H1). The "rain" (partitions with fluorescence between 80 and 40 RFU) observed in WT channel using gDNA and NTC templates (E1-F1), compared with the largely clear WT and Mut oligo templates (A1-D1), indicates the assay might also benefit from optimization for improved specificity (see Adjusting assay conditions section). NTC: No template control.

## Conclusion

Our dPCR LNA Mutation Assays provide sensitive and specific mutation detection results at the recommended conditions in the majority of situations however, in rare situations, minor adjustments might be necessary. According to the guidance provided here, some basic testing and adjustment to conditions to ensure the best performance of assays is outlined. The QIAcuityDx Four in Utility Mode and QIAcuityDx Universal MasterMix Kit have been specifically designed to flexibly support the use of a user's own assays and enable best performance in their specific workflows.

# Ordering Information

| Product | Contents | Cat. no. |
|---|---|---|
| QIAcuityDx Four | For nanoplate-based digital PCR diagnostic applications. | 911060 |
| QIAcuityDx Universal MasterMix Kit (1 mL; 5 mL) | QIAcuityDx Universal MasterMix, $MgCl_2$, 200mM, RNase-free Water | 260101; 260102 |
| QIAcuityDx Nanoplate 26k 24-well (10) | 10 QIAcuityDx Nanoplate 26k 24-well, 11 Nanoplate Seals | 260001 |
| QIAamp DSP Circulating NA Kit | For 50 preps: includes QIAamp Mini Columns, Buffers, Carrier RNA, QIAGEN Proteinase K, and Tubes | 61504 |
| dPCR LNA Mutation Assay (200) | Single tube containing ready-to-use 30x-concentrated assay with choice of FAM + HEX or Atto 550 + ROX detection dyes; sufficient for 200 dPCR reactions of 40 µl each | 250200 |

## References


1. Scimone, C., Pepe, F., Russo, G., Palumbo, L., Ball, G., Morel, P., Russo, A., Troncone, G., and Malapelle, U. Technical evaluation of a novel digital PCR platform for detecting EGFR/KRAS mutations in NSCLC archived plasma specimens. *J Liquid Biopsy* 2024; **3**:100133.
2. Quick-Start Protocol dPCR LNA Mutation Assays, 2020. https://geneglobe.qiagen.com/us/product-groups/dpcr-lna-mutation-assays?q=DMH0000085
3. QIAcuityDx User Manual, 2024
4. QIAcuityDx Universal MasterMix Kit Handbook, 2024.

Explore how QIAcuityDx can simplify your clinical testing workflows.
Scan the QR code or visit **qiagen.com/qiacuitydx** to learn more.

The QIAcuityDx Four is intended for in vitro diagnostic use. Product availability may differ from country to country based on regulations and approvals. Contact your country representative for further details.