Crystal Digital PCR® Assay

Information Sheet

For Research Use Only. Not for use in diagnostic procedures.

Product Name

NRAS (drop-off Q61) Crystal Digital PCR® Assay (R51016)

Description

Detected Targets

Targets	Sample Type	Detection Channels	Multiplex
NRAS Drop-off Q61	DNA	Blue/Green	2

The NRAS (drop-off Q61) Crystal Digital PCR® Assay is a 10X assay designed to detect and quantify mutations in codon Q61 of the NRAS gene using the Ruby Chip. NRAS is pivotal in regulating cell signaling pathways implicated in cancer development, notably melanoma and colorectal cancer.

Assay Configuration

The table below indicates with a “X” which channel(s) are used for each target in the assay:

Targets	Blue	Teal	Green	Yellow	Red	Infra-Red	Long-Shift
Wild-type (WT) NRAS Q61	X		X				
Mutant (MUT) NRAS Q61			X				

Components

NRAS (drop-off Q61) Crystal Digital PCR® Assay comprises two reagents: a pool of the assay specific primers and Crystal Flex Probes and a Positive Control. Please refer to the lot specific Certificate of Conformity for characterized concentration, available upon demand to Stilla’s Technical Support team at support-stilla@bio-rad.com.

Component Name	Reference	Concentration	Description
NRAS (drop-off Q61) Crystal Digital PCR® Assay	R51016	10X	Detects mutations in codon Q61 of the NRAS gene
NRAS Positive Control	R51016.PC0	10X	Contains: hgDNA, Synthetic NRAS mutants (Q61R, Q61K)

Thermocycling Programs

On the naica system:

Step		Ramp rate
Step 1	Partition for Ruby Chip	-
Step 2	Temperature 95°C for 180 seconds	1°C/sec
Step 3	Begin Loop for 60 Iterations	-
Step 3.1	Temperature 95°C for 15 seconds	1°C/sec
Step 3.2	Temperature 58°C for 30 seconds	1°C/sec
Step 4	Release for Ruby Chip	-

On the Nio Digital PCR:

Step		Ramp rate
Step 1	Partition for Ruby Chip	-
Step 2	Temperature 95°C for 180 seconds	1°C/sec
Step 3	Begin Loop for 60 Iterations	-
Step 3.1	Temperature 95°C for 15 seconds	2°C/sec
Step 3.2	Temperature 60°C for 30 seconds	2°C/sec
Step 4	Temperature 58°C for 300 seconds	1°C/sec
Step 5	Release for Ruby Chip	-

Data Acquisition

Download Nio dedicated technical files from bio-rad.com.

- NioProtocol_3C-60X-60°C-30s+58°C300s.nioprotocol (Nio Digital PCR)
- NioAssay_3C_NRAS_R51016.nioassay (Nio Digital PCR)

Download naica dedicated technical files from bio-rad.com.

- ScanningTemplate_Prism3_NRAS_R51016.ncx (3-color naica system)
- ScanningTemplate_Prism6_NRAS_R51016.ncx (6-color naica system)

Data Analysis

The following files are embedded in the dedicated scanning files listed above:

- CompensationMatrix_Prism3_NRAS_R51016.ncm (3-color naica system)
- UniversalCompMatrix_3C_Prism6-Nio.ncm (6-color naica® system, Nio Digital PCR)
- AnalysisConfiguration_NRAS_R51016.nca (all systems)

Consumables Required but Not Provided

- Ruby Chip (C16011)

- naica® PCR MIX 10X (R10106)
- Crystal Universal Reporters 3 (R41401 200 reactions, R41402 1000 reactions)
- Nuclease-free water

Instruction for PCR Mix Preparation

Specific instructions for preparing the PCR mix are given below.

Reagent Name	Initial Concentration	Final Concentration	Volume per reaction (µL)
naica® PCR MIX Buffer A ●	10x	1x	0.60
naica® PCR MIX Buffer B ●	100%	4%	0.24
Crystal Digital PCR® Assay ●	10x	1x	0.60
Crystal Universal Reporter Tube A ○	40x	1x	0.15
Nuclease-free water	NA	NA	Variable
Template DNA	**NA**	**NA**	**Variable**
or Positive Control ○	**10x**	**1x**	**0.60**
		Total reaction volume (µL)	***6.0***

DNA Digestion

DNA samples with ≥10 kb average length (e.g., genomic DNA) could be fragmented by restriction digestion before partitioning to ensure even distribution of the DNA template during partitioning. Restriction digestion is not required for highly fragmented DNA (e.g., FFPE DNA or circulating DNA). This step could improve assay performance and should be tested utilizing desired samples.

Care must be taken to use restriction enzymes that do not cut within the amplified sequence or the Crystal Flex Probes.

For a list of restriction enzymes compatible with a given Crystal Digital PCR® assay, contact our Technical Support team (support-stilla@bio-rad.com).

Loading Amount

For optimal performance, it is recommended not to exceed a chamber concentration (DNA concentration in the reaction mix) of 1,000 copies/µL. The performance of the assay at higher concentrations is not guaranteed and must be validated by the user.

Representative Data and Instructions for Analysis

Set thresholds for separating positive and negative populations on the 1D plots. To optimize the analysis of the drop-off system, the Blue threshold should be set just above the negative cluster, while the Green threshold should be set just below the positive cluster. Examples of results obtained on the 3-color naica system are given below.

Remark: The Blue threshold can be adjusted on each individual chamber to optimize its placement. In this case, it is recommended to adjust the threshold in the 2D-plots.

Wet lab testing was carried out using genomic hgDNA and H_2O as negative controls and a positive control consisting of hgDNA and 2 synthetic NRAS mutants (Q61K and Q61R). Synthetic NRAS mutants were also tested individually (Q61K, Q61L, Q61R) as well as with a Horizon standard composed of 50% mutant DNA (Q61H) and 50% wild-type DNA.

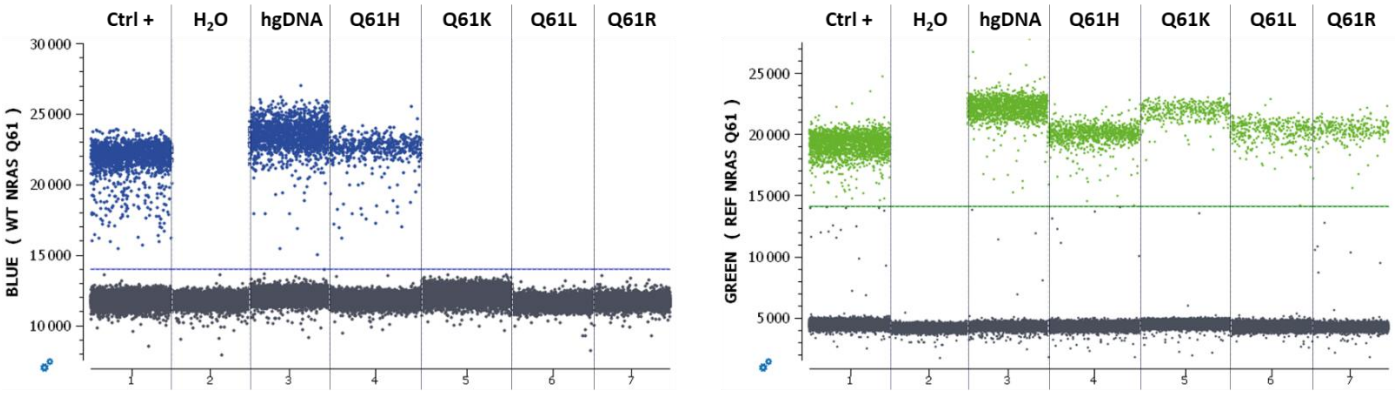


Figure 1: 1D plots obtained during wet lab testing on the 3-color naica system. *The Blue threshold is set just above the negative cluster, while the Green threshold is set just below the positive cluster.*

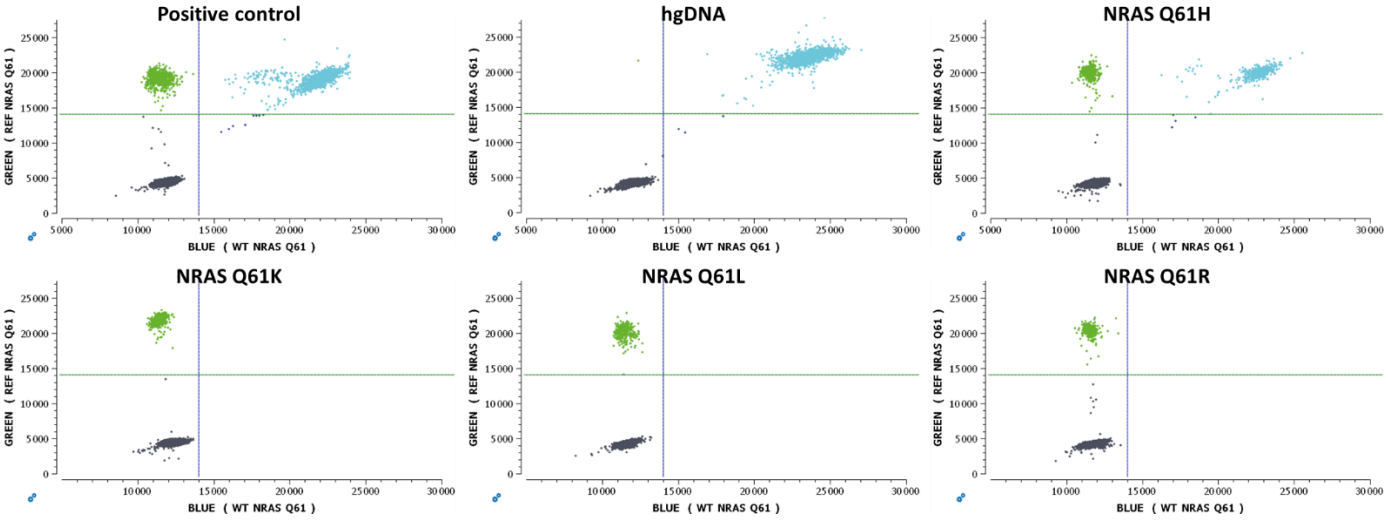
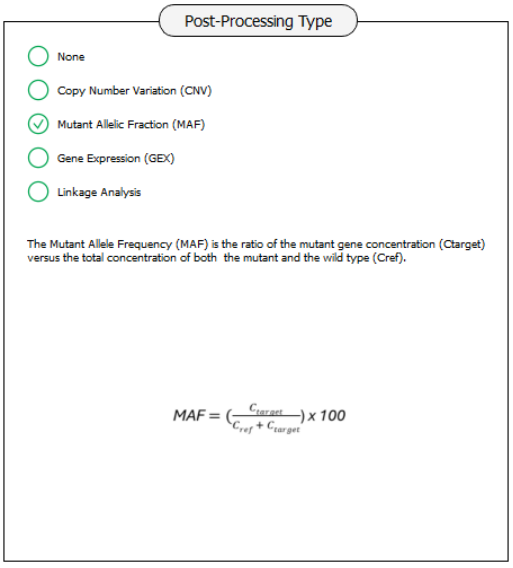


Figure 2: 2D plots obtained during wet lab testing on the 3-color naica system. *The Blue-Green double-positive population corresponds to wild-type DNA, while the Green single-positive population corresponds to mutated DNA.*

Post-Processing (only available with NioAnalyzer software)

To perform a post-processing analysis of the results, click on “Setup” in the “POST PROCESSING” menu and select the appropriate analysis: **Mutant Allelic Fraction (MAF)**. Follow specific instructions for this assay:



All populations should be added to processing, and “WT NRAS Q61” selected as reference.

Clicking on apply will launch the calculation. The values will be displayed in the “Results” tab.

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