Crystal Digital PCR® Assay

Information Sheet

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

Product Name

KRAS (G12A, G12C, G12D, G12V, drop-off G12-G13) Crystal Digital PCR® Assay (R51007)

Description

Detected Targets

Targets	Sample Type	Detection Channels	Multiplex
KRAS Drop-off G12-G13, G12A, G12C, G12D, G12V	DNA	Blue/Teal/Green/Yellow/Red/Infra-Red	6

The KRAS (G12A, G12C, G12D, G12V, drop-off G12-G13) Crystal Digital PCR® Assay is a 10X assay designed to detect and quantify mutations in codons 12 and 13 plus 4 mutations in the KRAS gene using the Ruby Chip.
KRAS is pivotal in regulating cell signaling pathways implicated in cancer development, notably melanoma and colorectal cancer.

Multiplexing Strategy: Color-Combination

This assay relies on the Color-Combination multiplexing strategy proprietary to Stilla Technologies, in which targets are detected, differentiated, and quantified by Crystal Digital PCR using 2 fluorophores.

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

Targets	Base changes	Blue	Teal	Green	Yellow	Red	Infra-Red	Long-Shift
Wild-type (WT) KRAS G12-G13	N/A	X	X					
Additional mutant (Add. MUT) KRAS G12-G13	N/A		X					
KRAS G12A	c.35G>C		X				X	
KRAS G12C	c.34G>T		X			X		
KRAS G12D	c.35G>A		X	X				
KRAS G12V	c.35G>T		X		X			

Remark: The mutants potentially detected by the drop-off system are mutations in addition to those targeted directly by the specific probes (G12A/C/D/V). Thus, if a mutant targeted by the system is detected (i.e. G12A), it will not be quantified by the drop-off system. Conversely, if a G12-G13 mutation other than G12A/C/D/V is detected, it will be quantified by the drop-off system (Population: Add. MUT KRAS G12-G13).

Components

KRAS (G12A, G12C, G12D, G12V, drop-off G12-G13) 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
KRAS (G12A, G12C, G12D, G12V, drop-off G12-G13) Crystal Digital PCR® Assay	R51007	10X	Detects mutations in KRAS codons G12-G13 and detects individually 4 mutations in the KRAS gene.
KRAS Positive Control	R51007.PC0	10X	Contains: hgDNA, Synthetic KRAS mutants (G12A, G12C, G12D, G12V)

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_6C-60X-60°C-30s+58°C300s.nioprotocol (Nio Digital PCR)
- NioAssay_6C_KRAS_R51007.nioassay (Nio Digital PCR)

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

- ScanningTemplate_Prism6_KRAS_R51007.ncx (6-color naica system)

Image Analysis

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

- CompensationMatrix_Prism6_KRAS_R51007.ncm (6-color naica system)
- CompensationMatrix_Nio_KRAS_R51007.ncm (Nio Digital PCR)
- AnalysisConfiguration_KRAS_R51007.nca (all systems)

Consumables Required but Not Provided

- Ruby Chip (C16011)
- naica® PCR MIX 10X (R10106)
- Universal Reporters 7 (R42401 200 reactions, R42402 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
Crystal Universal Reporter Tube B ●	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, the Teal threshold should be set **just below the positive cluster** and the Green/Yellow/Red/Infra-Red thresholds should be set at approximately equal distance from the positive and negative clusters. An incorrect threshold placement could lead to false positive results. The 2D-dot plots can be used to set the thresholds for each chamber (figure 1). Examples of results obtained on the Nio™+ system are given below (figure 2 and 3).

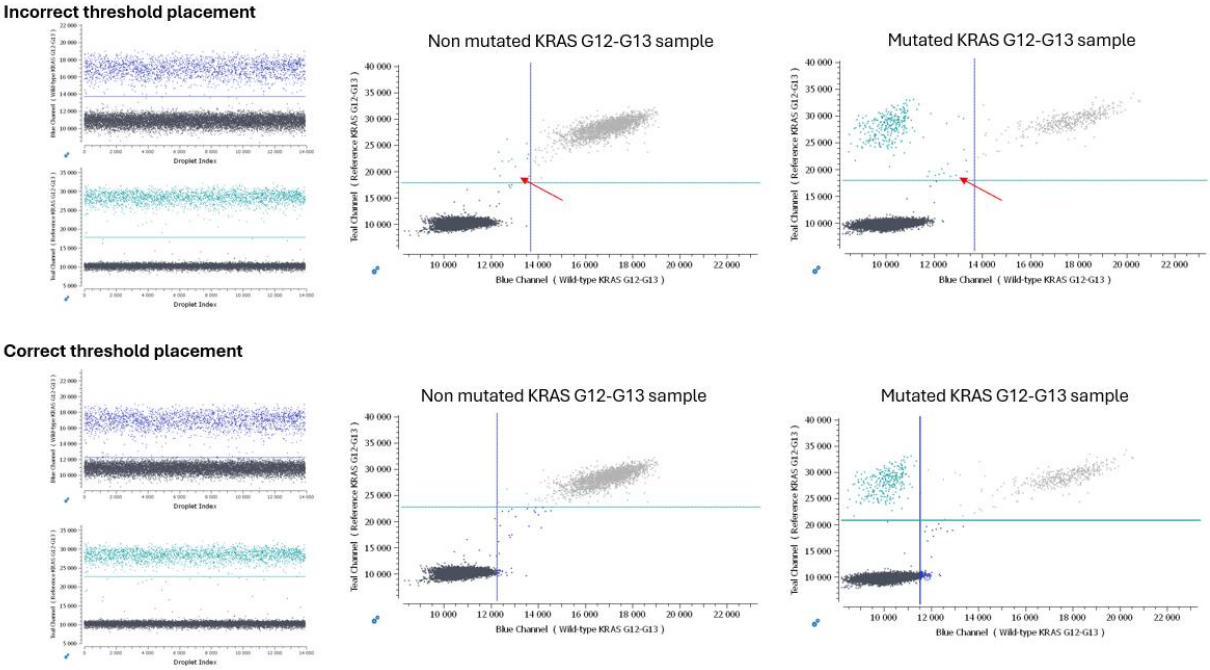


Figure 1: 1D and 2D dot plots obtained on the Nio+. *Droplets blue+teal positive are considered as KRAS G12-G13 non mutated, and droplets teal positive/blue negative are considered as mutated for KRAS G12-G13. With an incorrect placement of blue and teal thresholds, it could lead to false positive results for KRAS G12-G13 mutation (red arrow). By positioning the thresholds in this manner, it is possible for the blue threshold to be slightly overlapped on the negative droplet cluster. As no target is quantified only in blue, this will have no significant impact on quantification.*

Wet lab testing was carried out using genomic hgDNA as a negative control and a positive control consisting of hgDNA and 4 synthetic KRAS mutants (G12A, G12C, G12D, G12V). Synthetic KRAS mutants were also tested individually (G12A, G12C, G12D, G12V) as well as with Horizon standards composed of 50% mutant DNA (G12R or G12S) and 50% wild-type DNA.

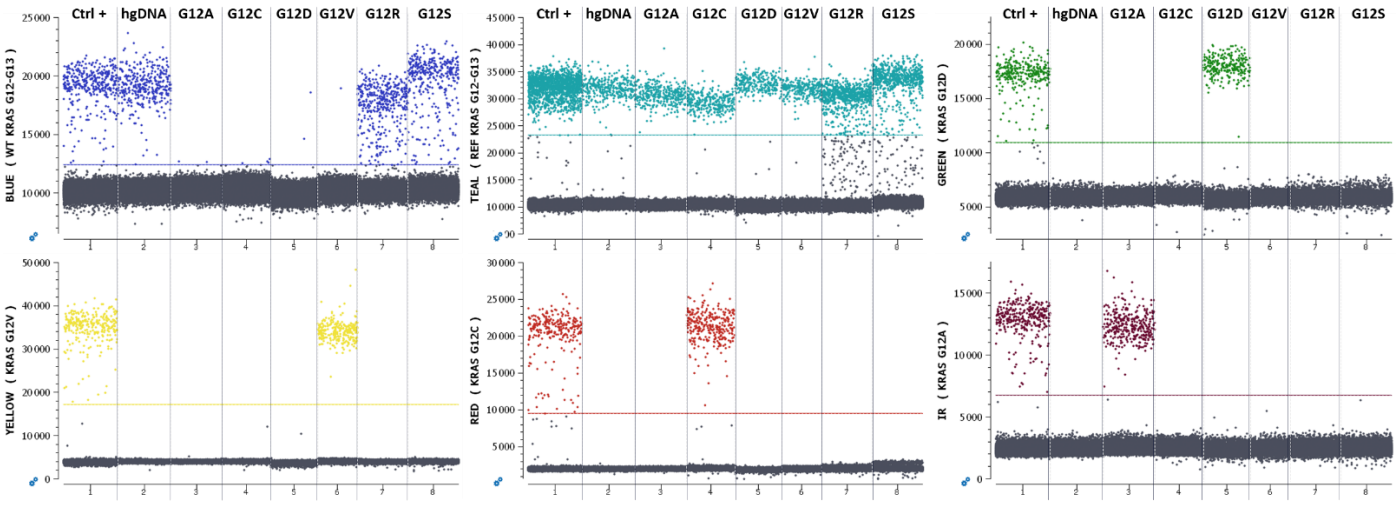


Figure 2: 1D plots obtained during wet lab testing on the Nio+. *The Blue threshold is set just above the negative cluster, the Teal threshold is set just below the positive cluster and the Green/Yellow/Red/Infra-Red thresholds are set at approximately equal distance from the positive and negative clusters.*

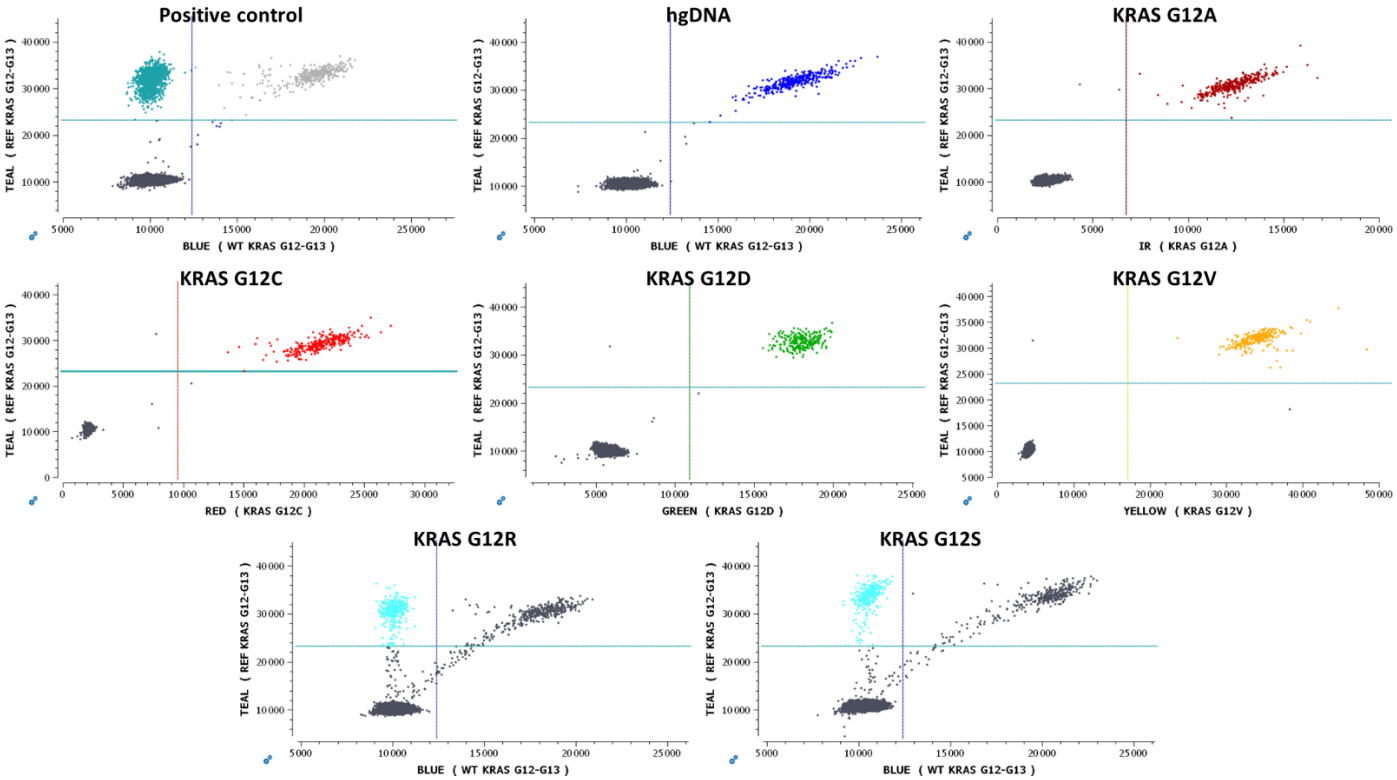
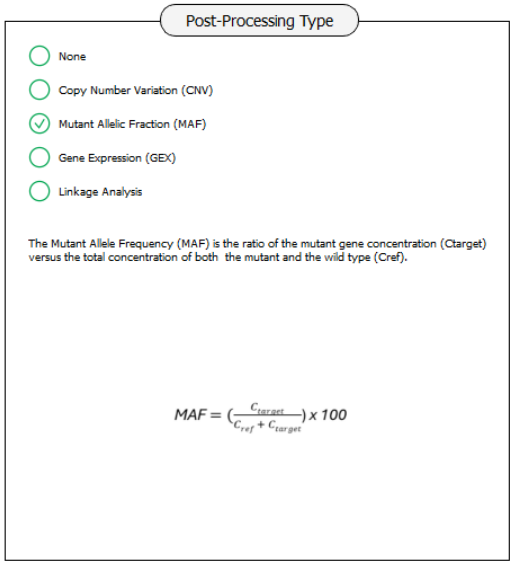
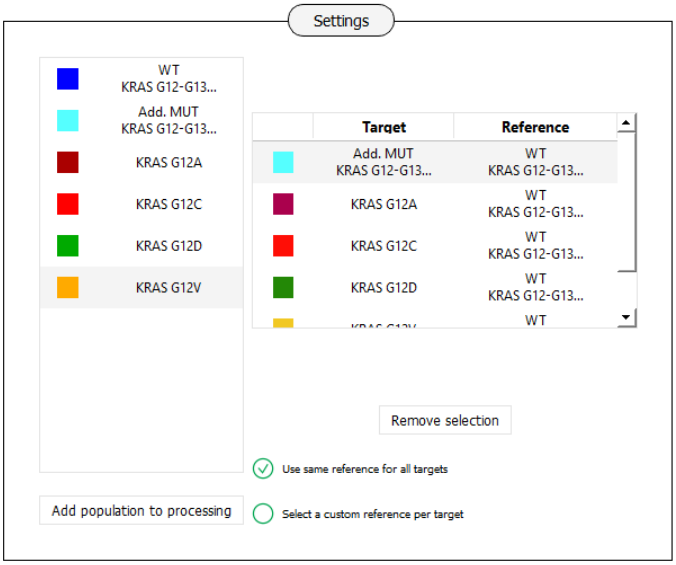


Figure 3: 2D plots obtained during wet lab testing on the Nio+. *The Blue-Teal double-positive population corresponds to wild-type DNA and the targeted mutants are characterized by a 2-colors signal: Teal + Mutant specific color (i.e. KRAS G12A = Teal/IR). The Teal single-positive population corresponds to additional G12-G13 mutated DNA such as G12R and G12S.*

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 KRAS G12-G13” selected as reference for all targets.

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

AIS_R51007_v3

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