

# Crystal Digital PCR® Assay

## Information Sheet

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

### Product Name

NRAS (G12D, drop-off G12-G13) Crystal Digital PCR® Assay (R51032)

### Description

#### Detected Targets

Targets	Sample Type	Detection Channels	Multiplex
NRAS G12D, drop-off G12-G13	DNA	Blue/Green/Red	3

The NRAS (G12D, drop-off G12-G13) Crystal Digital PCR® Assay is a 10X assay designed to detect and quantify mutations in codons 12 and 13 plus 1 mutation of the NRAS gene. NRAS 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(s) are used for each target in the assay:

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

Remark: The mutants potentially detected by the drop-off system are mutations in addition to NRAS G12D targeted directly by a specific probe. Thus, if NRAS G12D is detected, it will not be quantified by the drop-off system. Conversely, if a G12-G13 mutation other than G12D is detected, it will be quantified by the drop-off system (Population: Add. MUT NRAS G12-G13).

## Components

NRAS (G12D, 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](mailto:support-stilla@bio-rad.com).

Component Name	Reference	Concentration	Description
<b>NRAS (G12D, drop-off G12-G13) Crystal Digital PCR® Assay</b>	R51032	10X	Detects mutations in codons G12 and G13 of the NRAS gene, and individually detects 1 mutation in the NRAS gene
<b>NRAS Positive Control</b>	R51032.PC0	10X	Contains: hgDNA, Synthetic NRAS mutants (G12C, G12D, G13R)

## Thermocycling Programs

On the naica system:

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

On the Nio Digital PCR:

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

## Data Acquisition

Download Nio dedicated technical files from [bio-rad.com](http://bio-rad.com).

- NioProtocol\_3C-60X-60°C-30s+58°C300s.nioprotocol (Nio Digital PCR)
- NioAssay\_3C\_NRAS\_R51032.nioassay (Nio Digital PCR)

Download naica dedicated technical files from [bio-rad.com](http://bio-rad.com).

- ScanningTemplate\_Prism3\_NRAS\_R51032.ncx (3-color naica system)
- ScanningTemplate\_Prism6\_NRAS\_R51032.ncx (6-color naica system)

## Data Analysis

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

- CompensationMatrix\_Prism3\_NRAS\_R51032.ncm (3-color naica® system)
- CompensationMatrix\_Prism6\_NRAS\_R51032.ncm (6-color naica® system)
- CompensationMatrix\_Nio\_NRAS\_R51032.ncm (Nio™ Digital PCR)
- AnalysisConfiguration\_NRAS\_R51032.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
<b>Template DNA</b>		<b>NA</b>	<b>NA</b>	<b>Variable</b>
<i>or Positive Control</i>	○	10x	1x	0.60
<i>Total reaction volume (µL)</i>				<b>6.0</b>

## 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](mailto:support-stilla@bio-rad.com)).

## Loading Amount

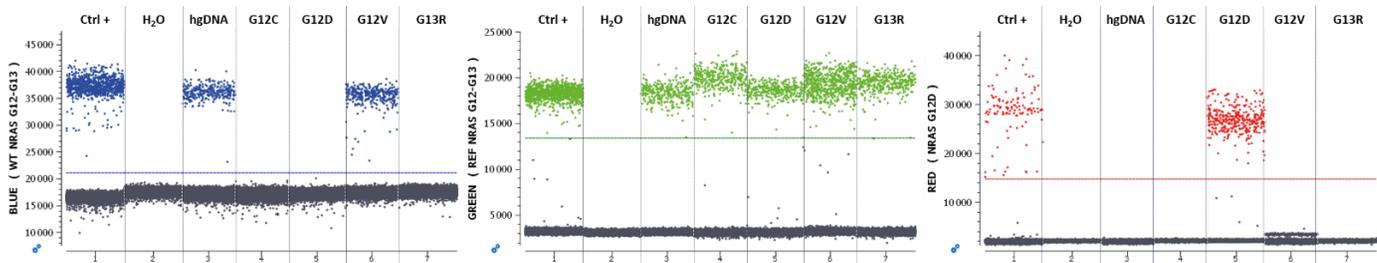
For optimal performance, it is recommended not to exceed a chamber concentration (DNA concentration in the reaction mix) of 1,000 copies/ $\mu\text{L}$ . The performance of the assay at higher concentrations is not guaranteed and must be validated by the user.

## Representative Data and Instructions for Analysis

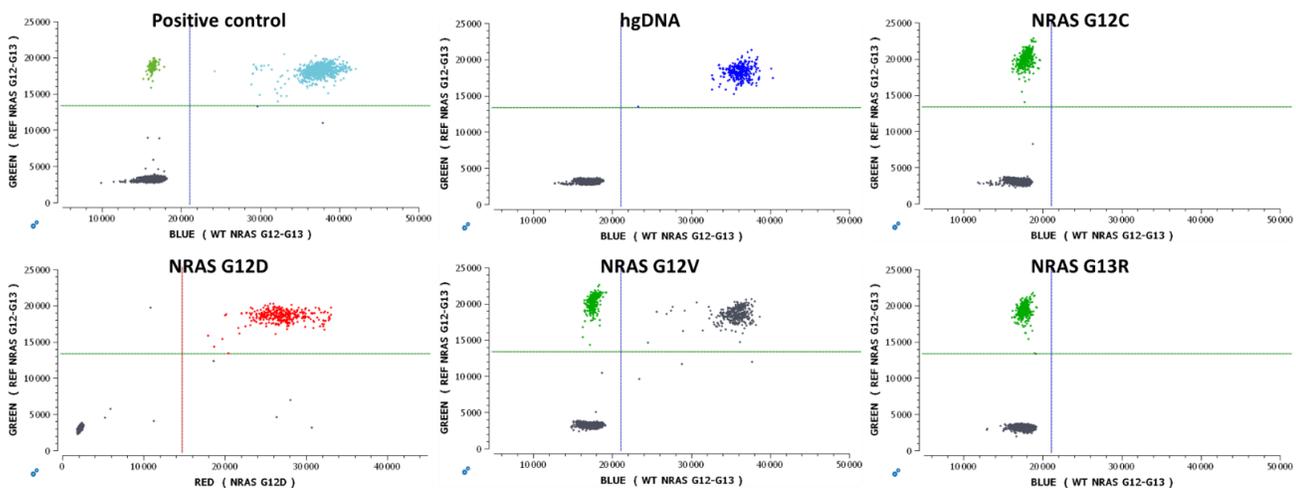
Set thresholds for separating positive and negative populations on the 1D plots. To optimize the analysis, the Blue threshold should be set just above the negative cluster, the Green threshold should be set just below the positive cluster and the Red threshold should be set at approximately equal distance from the positive and negative clusters. 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 as a negative control and a positive control consisting of hgDNA and 3 synthetic NRAS mutants (G12C, G12D, G13R). Synthetic NRAS mutants were also tested individually (G12C, G12D, G13R) as well as with Horizon standards composed of 50% mutant DNA (G12V) 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, the Green threshold is set just below the positive cluster and the Red threshold is set at approximately equal distance from the positive and negative clusters. Remark: a slight non-specific reaction of NRAS G12D probe on NRAS G12V may be observed in the red channel.



**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 Red-Green double-positive population corresponds to NRAS G12D. The Green single-positive population corresponds to additional G12-G13 mutated DNA such as G12C/V or G13R.

## 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:

Post-Processing Type

- None
- Copy Number Variation (CNV)
- Mutant Allelic Fraction (MAF)
- Gene Expression (GEX)
- Linkage Analysis

The Mutant Allele Frequency (MAF) is the ratio of the mutant gene concentration (C<sub>target</sub>) versus the total concentration of both the mutant and the wild type (C<sub>ref</sub>).

$$MAF = \left( \frac{C_{target}}{C_{ref} + C_{target}} \right) \times 100$$

Settings

	Target	Reference
<input checked="" type="checkbox"/> WT NRAS G12-G13		
<input checked="" type="checkbox"/> Add. MUT NRAS G12-G13	Add. MUT NRAS G12-G13	WT NRAS G12-G13
<input checked="" type="checkbox"/> NRAS G12D	NRAS G12D	WT NRAS G12-G13

Use same reference for all targets

Select a custom reference per target

All populations should be added to processing, and “WT NRAS G12-G13” selected as reference.

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

AIS\_R51032\_v3



Stilla Technologies  
F-94800 Villejuif, FRANCE

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