

# Crystal Digital PCR® Assay

## Information Sheet

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

## Product Name

NRAS (G12, G12D) Crystal Digital PCR® Assay (R51033)

## Description

### Detected Targets

| Targets        | Sample Type | Detection Channels | Multiplex |
|----------------|-------------|--------------------|-----------|
| NRAS G12, G12D | DNA         | Blue/Red           | 2         |

The NRAS (G12, G12D) Crystal Digital PCR® Assay is a 10X assay designed to detect and quantify 1 mutation in 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                        | Base changes | Blue | Teal | Green | Yellow | Red | Infra-Red | Long-Shift |
|--------------------------------|--------------|------|------|-------|--------|-----|-----------|------------|
| Wild-type (WT)<br>NRAS G12-G13 | N/A          | X    |      |       |        |     |           |            |
| NRAS G12D                      | c.35G>A      |      |      |       |        | X   |           |            |

### Components

NRAS (G12, G12D) 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  |
|--|------------|---------------|--|
| NRAS (G12, G12D)<br>Crystal Digital PCR® Assay | R51033     | 10X           | Detects 1 mutation in the NRAS gene                        |
| NRAS Positive Control                          | R51033.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\_R51033.nioassay (Nio Digital PCR)

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

- ScanningTemplate\_Prism3\_NRAS\_R51033.ncx (3-color naica system)
- ScanningTemplate\_Prism6\_NRAS\_R51033.ncx (6-color naica system)

## Data Analysis

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

- CompensationMatrix\_Prism3\_NRAS\_R51033.ncm (3-color naica system)
- CompensationMatrix\_Prism6\_NRAS\_R51033.ncm (6-color naica system)
- CompensationMatrix\_Nio\_NRAS\_R51033.ncm (Nio Digital PCR)
- AnalysisConfiguration\_NRAS\_R51033.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  $\geq 10$  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/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, the thresholds should be set at approximately equal distance from the positive and negative clusters. Examples of results obtained on the Nio™+ are given below. Remark: The threshold can be adjusted on each individual chamber to optimize its placement.

Wet lab testing was carried out using genomic hgDNA as a negative control and a positive control consisting of hgDNA and synthetic NRAS mutants (G12C, G12D, G13R). Synthetic NRAS mutants were also tested individually (G12C, G12D, G13R).

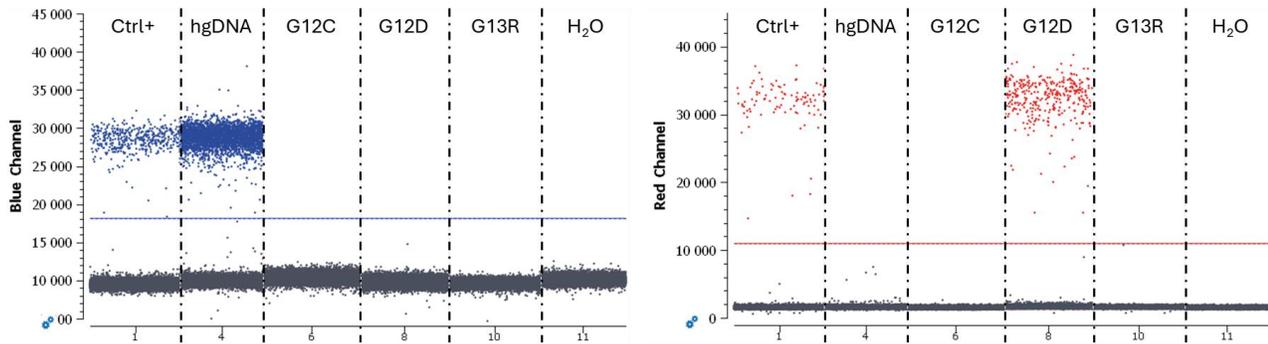


Figure 1: 1D plots obtained during wet lab testing on the Nio+. The thresholds are set at approximately equal distance from the positive and negative clusters.

### 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

■ B\_NRAS G12WT

■ R\_NRAS G12D

|   | Target      | Reference    |
|---|-------------|--------------|
| ■ | R_NRAS G12D | B_NRAS G12WT |

[Remove selection](#)

Use same reference for all targets  
 Select a custom reference per target

[Add population to processing](#)

All populations should be added to processing, and “B\_NRAS G12 WT” selected as reference.

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

AIS\_R51033\_v3



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