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 for download at the Technical Resources section of the Stilla Technologies website.

Component Name	Reference	Concentration	Description
NRAS (G12D, drop-off G12-G13) Crystal Digital PCR® Assay	R51032	10X	Detects mutations in codons G12 and G13 of the NRAS gene, and individually detects 1 mutation in the NRAS gene
NRAS Positive Control	R51002.PC0	10X	Contains: hgDNA, Synthetic NRAS mutants (G12C, G12D, G13R)

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	-

Image Acquisition

Download the dedicated scanning file from the Technical Resources section of the Stilla Technologies website:

- ScanningTemplate_Prism3_NRAS_R51032.ncx (3-color naica® system)
- ScanningTemplate_Prism6_NRAS_R51032.ncx (6-color naica® system)
- NioProtocol_3C-60X-60°C-30s.nioprotocol (Nio™ Digital PCR)
- NioAssay_3C_NRAS_R51032.nioassay (Nio™ Digital PCR)

Image 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
Template DNA		**NA**	**NA**	**Variable**
or Positive Control	⚪	*10x*	*1x*	*0.60*
Total reaction volume (µL)				*6.0*

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.
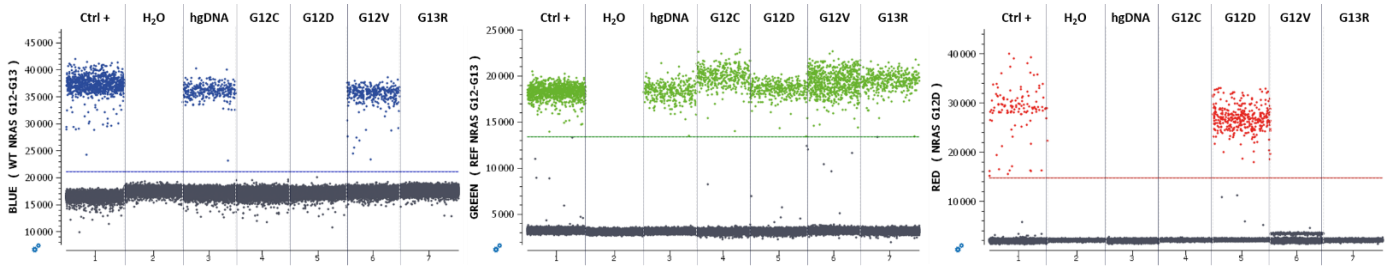
Figure 1: 1D plots obtained during wet lab testing on the 3-color naica® system. The the Blue threshold is set just above the negative cluster, the Green threshold is set just below the positive cluster and the Red threshold is set at approximately equal distance from the positive and negative clusters. Remark: a slight non-specific reaction of NRAS G12D probe on NRAS G12V may be observed in the red channel.
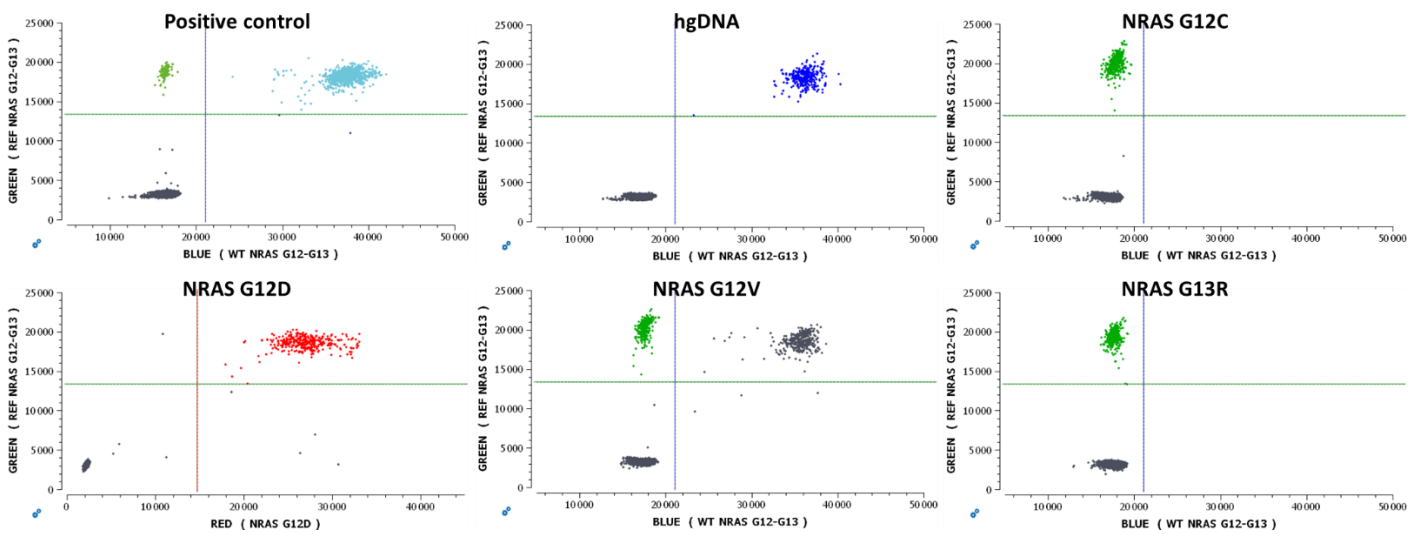

Figure 2: 2D plots obtained during wet lab testing on the 3-color naica® system. The Blue-Green double-positive population corresponds to wild-type DNA while the Red-Green double-positive population corresponds to NRAS G12D. The Green single-positive population corresponds to additional G12-G13 mutated DNA such as G12C//V or G13R.

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