Crystal Digital PCR® Assay

Information Sheet

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

Product Name

NRAS (Q61K, drop-off Q61) Crystal Digital PCR® Assay (R51018)

Description

Detected Targets

Targets	Sample Type	Detection Channels	Multiplex
NRAS Q61K, drop-off Q61	DNA	Blue/Green/Red	3

The NRAS (Q61K, drop-off Q61) Crystal Digital PCR® Assay is a 10X assay designed to detect and quantify mutations in codon Q61 plus 1 mutation 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.

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 Q61	N/A	X		X				
Additional mutant (Add. MUT) NRAS Q61	N/A			X				
NRAS Q61K	c.181C>A			X		X		

Remark: The mutants potentially detected by the drop-off system are mutations in addition to NRAS Q61K targeted directly by a specific probe. Thus, if NRAS Q61K is detected, it will not be quantified by the drop-off system. Conversely, if a Q61 mutation other than Q61K is detected, it will be quantified by the drop-off system (Population: Add. MUT NRAS Q61).

Components

NRAS (Q61K, 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 for download at the Technical Resources section of the Stilla Technologies website.

Component Name	Reference	Concentration	Description
NRAS (Q61K, drop-off Q61) Crystal Digital PCR® Assay	R51018	10X	Detects mutations in codon Q61 of the NRAS gene and individually detects 1 mutation in 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	-

Image Acquisition

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

- ScanningTemplate_Prism3_NRAS_R51018.ncx (3-color naica® system)
- ScanningTemplate_Prism6_NRAS_R51018.ncx (6-color naica® system)
- NioProtocol_3C-60X-60°C-30s.nioprotocol (Nio™ Digital PCR)
- NioAssay_3C_NRAS_R51018.nioassay (Nio™ Digital PCR)

Image Analysis

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

- CompensationMatrix_Prism3_NRAS_R51018.ncm (3-color naica® system)
- CompensationMatrix_Prism6_NRAS_R51018.ncm (6-color naica® system)
- CompensationMatrix_Nio_NRAS_R51018.ncm (Nio™ Digital PCR)
- AnalysisConfiguration_NRAS_R51018.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 of the drop-off system, 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 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.

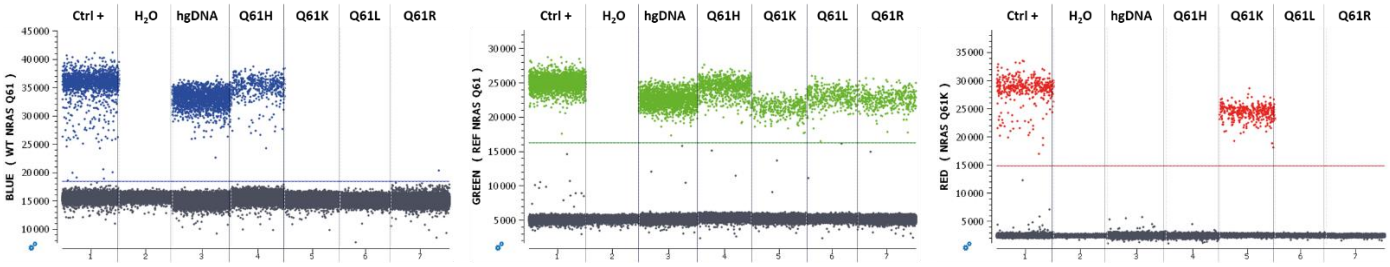

Figure 1: 1D plots obtained during wet lab testing on the 3-color naica® system. *The Blue threshold is set just above the negative cluster, the Green threshold is set just below the positive cluster and the Red threshold is set at approximately equal distance from the positive and negative clusters.*

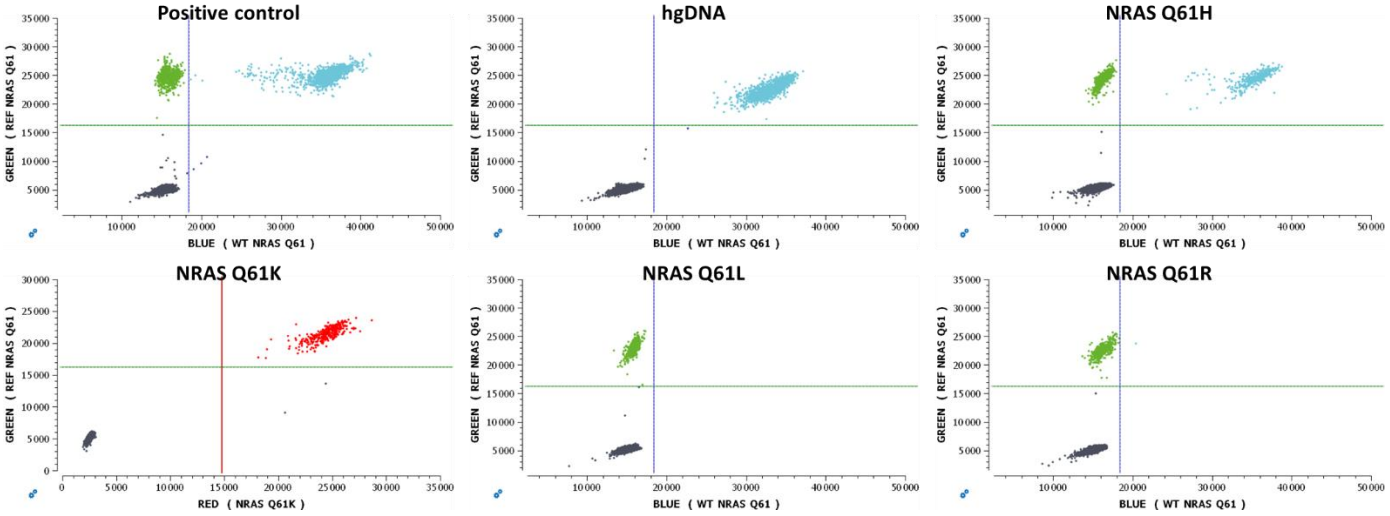

Figure 2: 2D plots obtained during wet lab testing on the 3-color naica® system. *The Blue-Green double-positive population corresponds to wild-type DNA while the Red-Green double-positive population corresponds to NRAS Q61K. The Green single-positive population corresponds to additional Q61 mutated DNA such as Q61H/L/R.*

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