Crystal Digital PCR® Assay

Information Sheet

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

Product Name

NRAS (Q61, Q61R) Crystal Digital PCR® Assay (R51017)

Description

Detected Targets

Targets	Sample Type	Detection Channels	Multiplex
NRAS Q61-Q61R	DNA	Blue/Green	2

The NRAS (Q61, Q61R) 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) NRAS Q61	N/A	X						
NRAS Q61R	c.182A>G			X				

Components

NRAS (Q61, Q61R) 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 Q61-Q61R Crystal Digital PCR® Assay	R51017	10X	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_R51017.ncx (3-color naica® system)
- ScanningTemplate_Prism6_NRAS_R51017.ncx (6-color naica® system)
- NioProtocol_3C-60X-60°C-30s.nioprotocol (Nio™ Digital PCR)
- NioAssay_3C_NRAS_R51017.nioassay (Nio™ Digital PCR)

Image Analysis

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

- CompensationMatrix_Prism3_NRAS_R51017.ncm (3-color naica® system)
- UniversalCompMatrix_3C_Prism6-Nio.ncm (6-color naica® system, Nio™ Digital PCR)
- AnalysisConfiguration_NRAS_R51017.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 and the green threshold should be set just at approximately equal distance from the positive and negative clusters. Examples of results obtained on the 3-color naica® system are given below.

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.

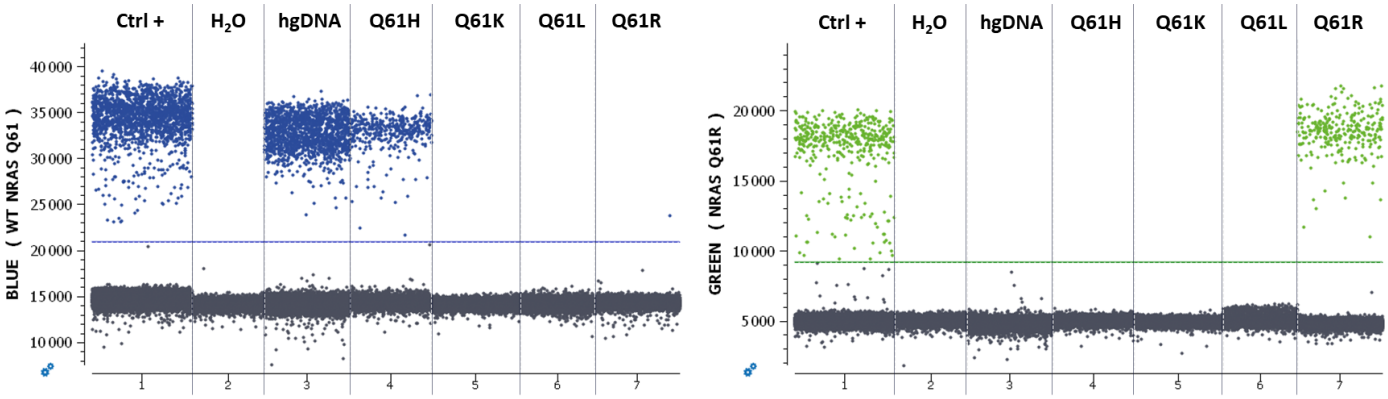

Figure 1: 1D plots obtained during wet lab testing on the 3-color naica® system.

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