Crystal Digital PCR® Assay

Information Sheet

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

Product Name

BRAF/KRAS/NRAS Crystal Digital PCR® Assay (R51006)

Description

Detected Targets

Targets	Sample Type	Detection Channels	Multiplex
BRAF (drop-off V600-K601) / KRAS (drop-off G12-G13) / NRAS (drop-off G12-G13)	DNA	Blue/Teal/Green/ Yellow/Red/Infra-Red	6

The BRAF/KRAS/NRAS Crystal Digital PCR® Assay is a 10X assay designed to detect and quantify mutations in BRAF (codons V600 and K601), KRAS (codons G12 and G13), and NRAS (codons G12 and G13) genes using the Ruby Chip and based on the drop-off approach (refer to the technical note "Quantify Drop-off for digital PCR assays with Crystal Miner", https://www.stillatechnologies.com/). BRAF, KRAS, and NRAS genes are pivotal in regulating cell signaling pathways implicated in cancer development.

Assay configuration

The table below indicates with a "X" which channel(s) are used for each target in the assay:

Targets	Blue	Teal	Green	Yellow	Red	Infra-Red	Long-Shift
Wild-type (WT) KRAS G12-G13	X	X					
Mutant (MUT) KRAS G12-G13	X						
Wild-type (WT) NRAS G12-G13			X			X	
Mutant (MUT) NRAS G12-G13			X				
Wild-type (WT) BRAF V600-K601				X	X		
Mutant (MUT) BRAF V600-K601				X			

Components

BRAF/KRAS/NRAS 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
BRAF/KRAS/NRAS Crystal Digital PCR® Assay	R51006	10X	Detects mutations in BRAF codons V600 and K601, KRAS codons G12 and G13, and NRAS codons G12 and G13.
BRAF/KRAS/NRAS Positive Control	R51006.PC0	10X	Contains: hgDNA, Synthetic mutants (BRAF V600E, KRAS G12C, NRAS G12D)

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 60°C for 60 seconds	1°C/sec
Step 4	Temperature 58°C for 300 seconds	1°C/sec
Step 5	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 62°C for 60 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_Prism6_BRAF-KRAS-NRAS_R51006.ncx (6-color naica® system)
- NioProtocol_6C-60X-62°C-60s.nioprotocol (Nio™ Digital PCR)
- NioAssay_6C_BRAF-KRAS-NRAS_R51006.nioassay (Nio™ Digital PCR)

Image Analysis

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

- CompensationMatrix_Prism6_BRAF-KRAS-NRAS_R51006.ncm (6-color naica® system)
- CompensationMatrix_Nio_BRAF-KRAS-NRAS_R51006.ncm (Nio™ Digital PCR)
- 2DplotsDefinition_BRAF-KRAS-NRAS_R51006.ncp (all systems)
- AnalysisConfiguration_Prism6_BRAF-KRAS-NRAS_R51006_Polygons.nca (6-color naica® system)
- AnalysisConfiguration_Nio_BRAF-KRAS-NRAS_R51006_Polygons.nca (Nio™ Digital PCR)

Consumables Required but Not Provided

- Ruby Chip (C16011)
- naica® PCR MIX 10X (R10106)
- Universal Reporters 7 (R42401 200 reactions, R42402 1000 reactions)
- Nuclease-free water

Instruction for PCR Mix Preparation

Specific instructions for preparing the PCR mix are given below.

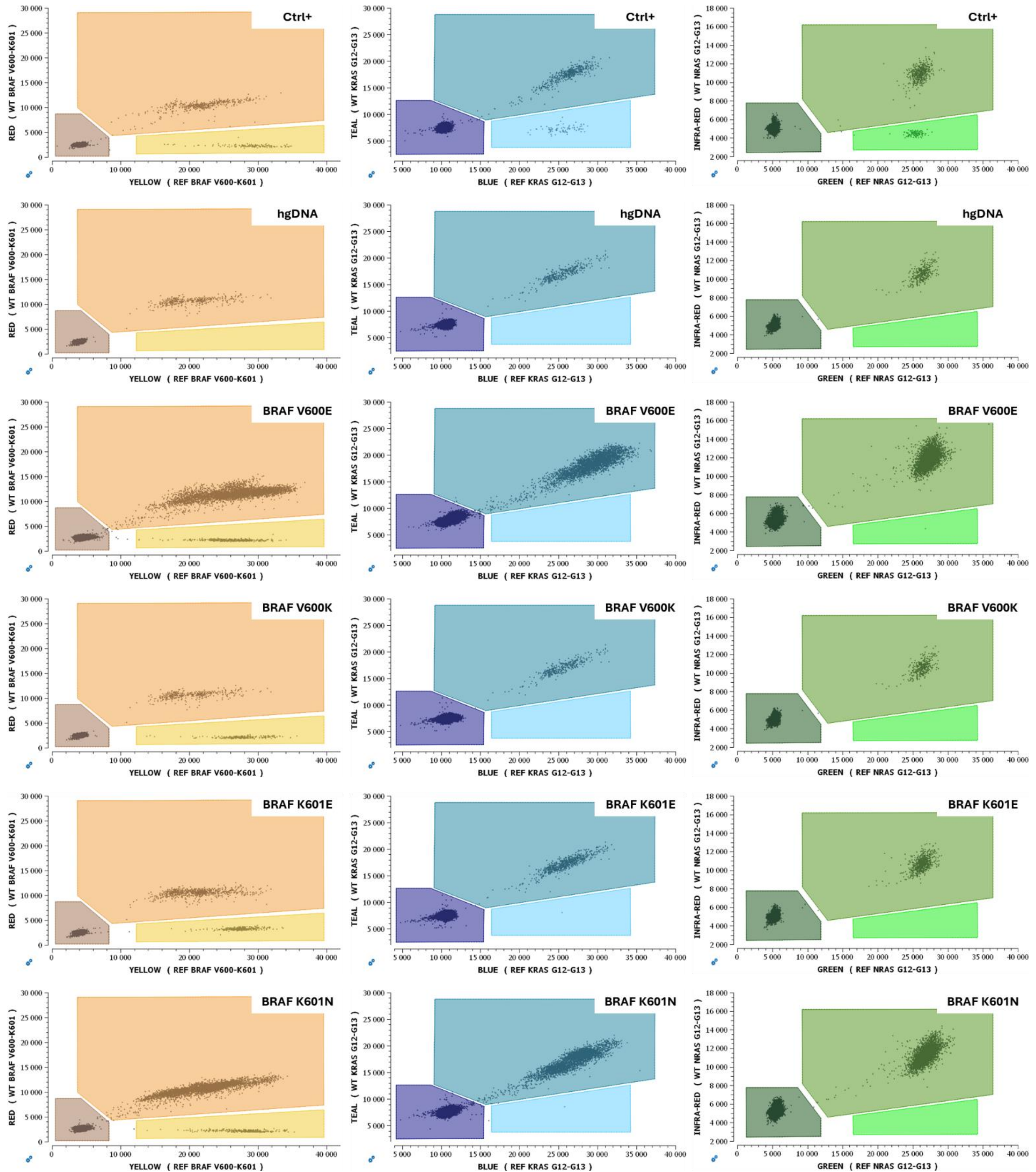
Reagent Name		Initial Concentration	Final Concentration	Volume per reaction (µL)
naica® PCR MIX Buffer A	●	10x	1x	0.60
naica® PCR MIX Buffer B	●	100%	4%	0.24
Crystal Digital PCR® Assay	●	10x	1x	0.60
Crystal Universal Reporter Tube A	●	40x	1x	0.15
Crystal Universal Reporter Tube B	●	40x	1x	0.15
Nuclease-free water		NA	NA	Variable
Template DNA		**NA**	**NA**	**Variable**
or Positive Control	○	**10x**	**1x**	**0.60**
			Total reaction volume (µL)	***6.0***

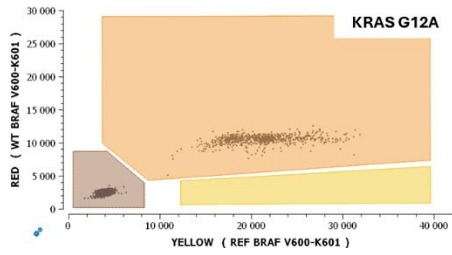
Representative Data and Instructions for Analysis

In the menu “Analyze data, Plots & Populations”, view the results in 2D dot plot. Check or manually adjust the position of the polygons for each target population according to the Positive Control. If needed, select “individual per chamber” in the thresholding mode to adjust the polygons for each sample. Examples of results obtained on the 6-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 3 synthetic mutants (BRAF V600E, KRAS G12C and NRAS G12D). Synthetic mutants were also tested individually (BRAF V600E/V600K/K601E/K601N, KRAS G12A/G12C/G12/G12V, NRAS G12C/G12D/G13R) as well

as with Horizon standards composed of 50% mutant DNA (KRAS G12R or KRAS G12S or NRAS G12V) and 50% wild-type DNA.




KRAS G12A
RED (WT BRAF V600-K601)
YELLOW (REF BRAF V600-K601)
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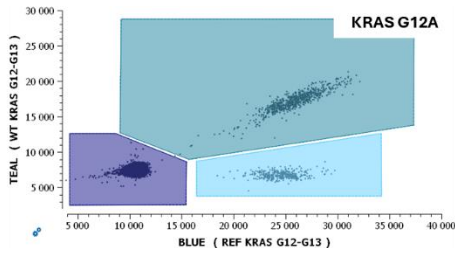
KRAS G12A
TEAL (WT KRAS G12-G13)
BLUE (REF KRAS G12-G13)
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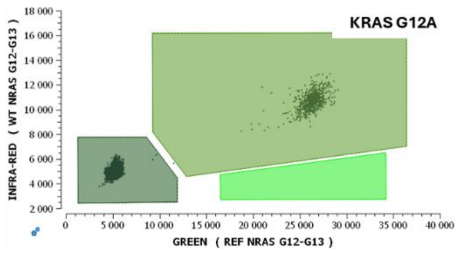
KRAS G12A
INFRA-RED (WT NRAS G12-G13)
GREEN (REF NRAS G12-G13)
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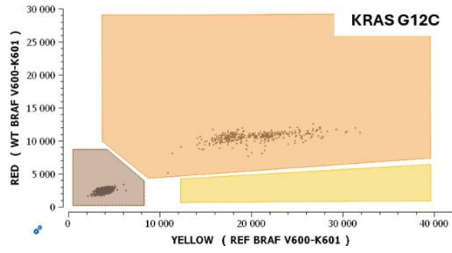
KRAS G12C
RED (WT BRAF V600-K601)
YELLOW (REF BRAF V600-K601)
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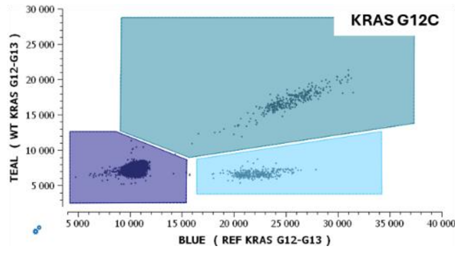
KRAS G12C
TEAL (WT KRAS G12-G13)
BLUE (REF KRAS G12-G13)
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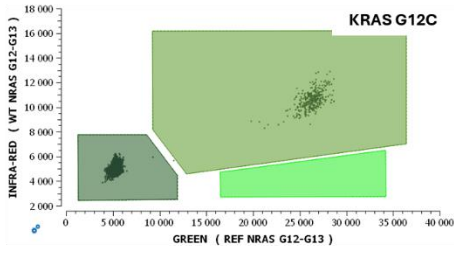
KRAS G12C
INFRA-RED (WT NRAS G12-G13)
GREEN (REF NRAS G12-G13)
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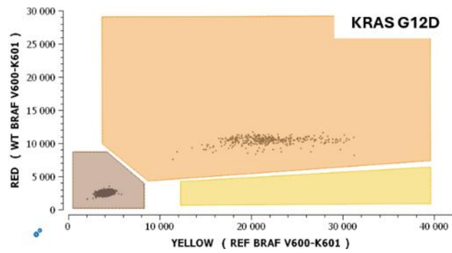
KRAS G12D
RED (WT BRAF V600-K601)
YELLOW (REF BRAF V600-K601)
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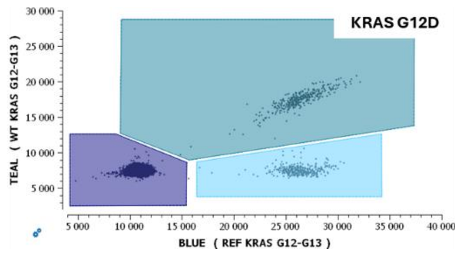
KRAS G12D
TEAL (WT KRAS G12-G13)
BLUE (REF KRAS G12-G13)
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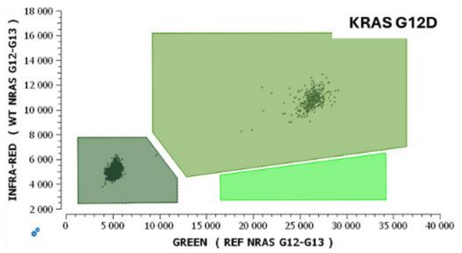
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GREEN (REF NRAS G12-G13)
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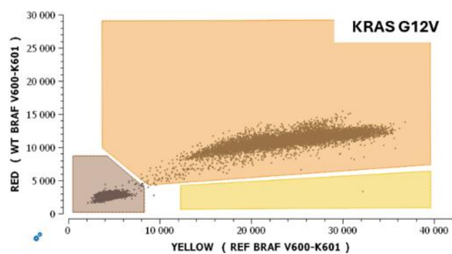
KRAS G12V
RED (WT BRAF V600-K601)
YELLOW (REF BRAF V600-K601)
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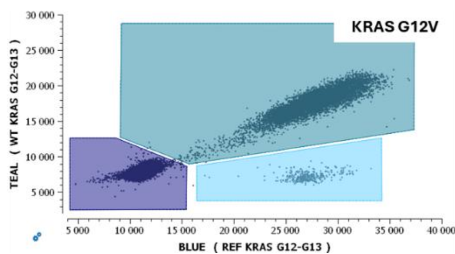
KRAS G12V
TEAL (WT KRAS G12-G13)
BLUE (REF KRAS G12-G13)
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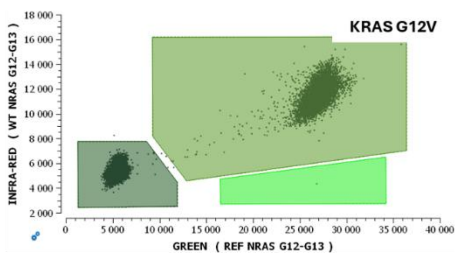
KRAS G12V
INFRA-RED (WT NRAS G12-G13)
GREEN (REF NRAS G12-G13)
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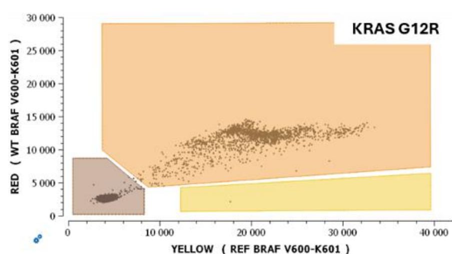
KRAS G12R
RED (WT BRAF V600-K601)
YELLOW (REF BRAF V600-K601)
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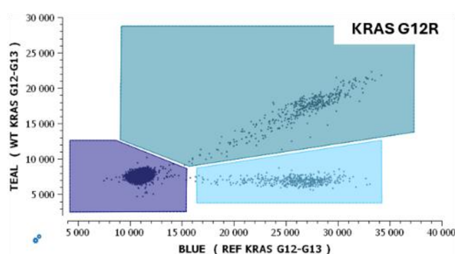
KRAS G12R
TEAL (WT KRAS G12-G13)
BLUE (REF KRAS G12-G13)
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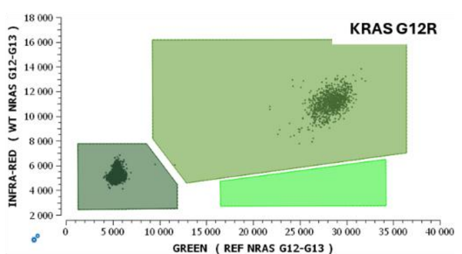
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GREEN (REF NRAS G12-G13)
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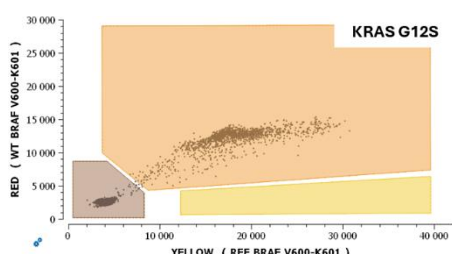
KRAS G12S
RED (WT BRAF V600-K601)
YELLOW (REF BRAF V600-K601)
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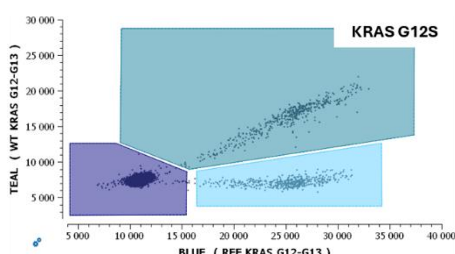
KRAS G12S
TEAL (WT KRAS G12-G13)
BLUE (REF KRAS G12-G13)
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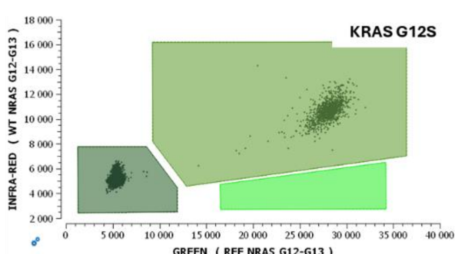
KRAS G12S
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GREEN (REF NRAS G12-G13)
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Figure 1: 2D plots obtained during wet lab testing on the 6-color naica® system. *The polygons should be adjusted for each target population and for each sample.*

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