Crystal Digital PCR® Assay

Information Sheet

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

Product Name

BRAF (drop-off V600-K601) Crystal Digital PCR® Assay (R51019)

Description

Detected Targets

Targets	Sample Type	Detection Channels	Multiplex
BRAF (drop-off V600-K601)	DNA	Blue/Green	2

The BRAF (drop-off V600-K601) Crystal Digital PCR® Assay is a 10X assay designed to detect and quantify mutations codons V600 and K601 of the BRAF gene. BRAF is pivotal in regulating cell signaling pathways implicated in cancer development, notably melanoma, colorectal cancer and thyroid cancer, through its role in the MAPK/ERK pathway.

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) BRAF V600-K601	X		X				
Mutant (MUT) BRAF V600-K601			X				

Components

BRAF (drop-off V600-K601) 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 (drop-off V600-K601) Crystal Digital PCR® Assay	R51019	10X	Detects mutations in codons V600 and K601 of the BRAF gene
KRAS Positive Control	R51019.PC0	10X	Contains: hgDNA, Synthetic BRAF mutants (V600E, V600K, K601E, K601N)

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 30 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 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_BRAF_R51019.ncx (3-color naica® system)
- ScanningTemplate_Prism6_BRAF_R51019.ncx (6-color naica® system)
- NioProtocol_3C-60X-60°C-30s.nioprotocol (Nio™ Digital PCR)
- NioAssay_3C_BRAF_R51019.nioassay (Nio™ Digital PCR)

Image Analysis

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

- CompensationMatrix_Prism3_BRAF_R51019.ncm (3-color naica® system)
- UniversalCompMatrix_3C_Prism6-Nio.ncm (6-color naica® system, Nio™ Digital PCR)
- AnalysisConfiguration_BRAF_R51019.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, while the green threshold should be set just below the positive cluster. Examples of results obtained on the Nio™+ 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 4 synthetic BRAF mutants (V600E, V600K, K601E, K601N). Synthetic BRAF mutants were also tested individually (V600E, V600K, K601E, K601N).

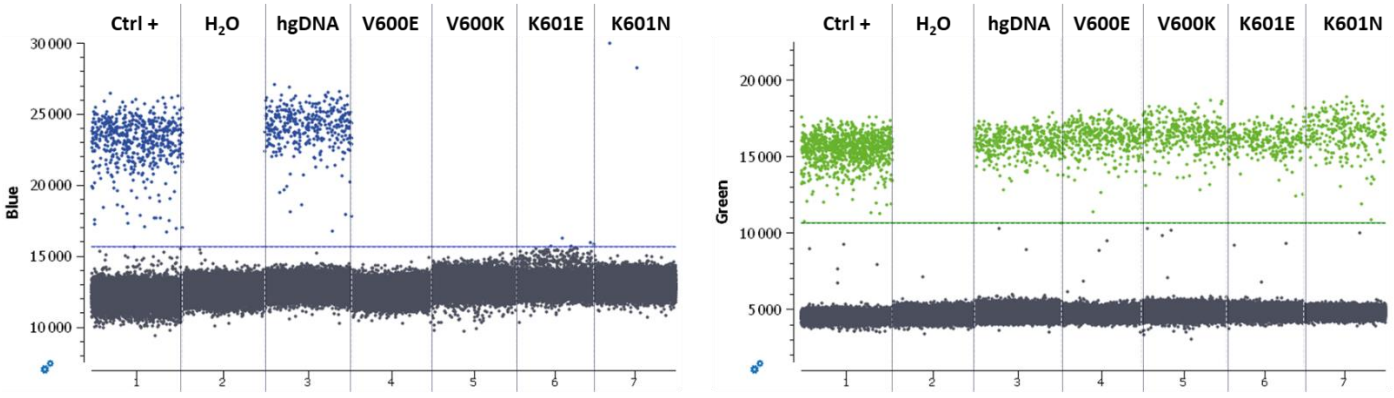

Figure 1: 1D plots obtained during wet lab testing on the 3-color naica® system. *The blue threshold is set just above the negative cluster, while the green threshold is set just below the positive cluster. Remark: a slight non-specific reaction of BRAF V600-K601 WT probe on BRAF K601E can be observed in the Blue channel.*

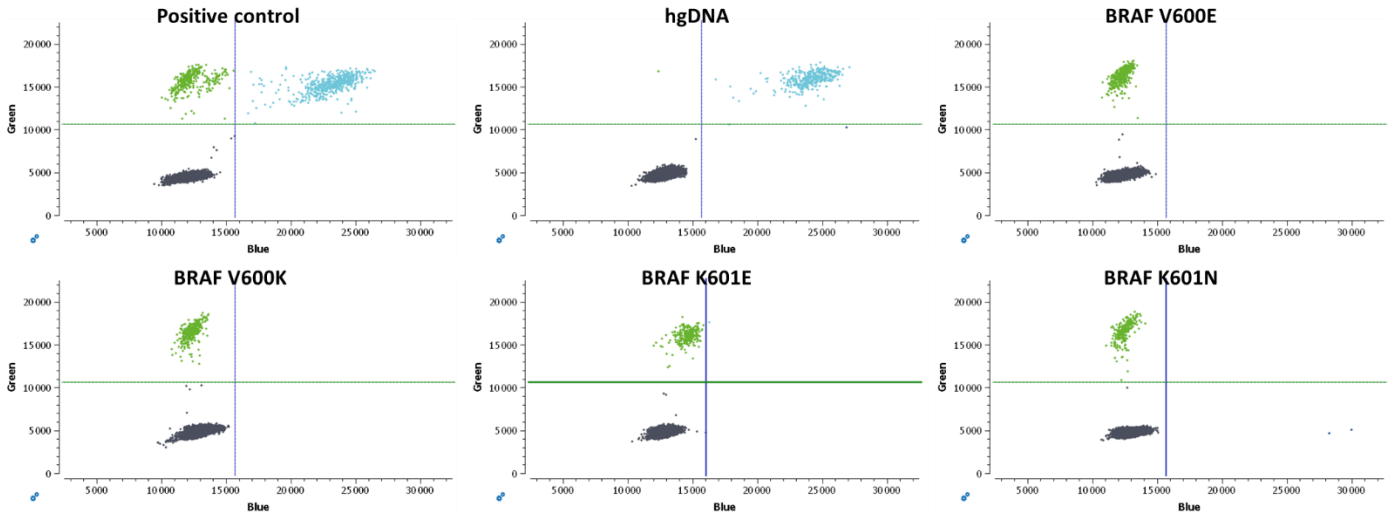

Figure 2: 2D plots obtained during wet lab testing on the Nio™+. *The Blue-Green double-positive population corresponds to wild-type DNA, while the Green single-positive population corresponds to mutated DNA.*

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