Crystal Digital PCR® Assay

Information Sheet

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

Product Name

Pan-Cancer 1 (EGFR, KRAS, NRAS, BRAF, IDH1) Crystal Digital PCR® Assay (R51041)

Description

Detected Targets

Targets	Sample Type	Detection Channels	Multiplex
EGFR deletion Exon19, EGFR L858R KRAS G12C, KRAS G12D NRAS Q61R BRAF V600E IDH1 R132H	DNA	Blue/Teal/Green/ Yellow/Red/Infra-Red	8

The Pan-Cancer1 (EGFR, KRAS, NRAS, BRAF, IDH1) Crystal Digital PCR® Assay is a 10X assay designed to detect and quantify mutations in multiple genes using the Ruby Chip. These genes play major roles in regulating cell signaling pathways, and their mutations drive tumor development. Numerous targeted therapies are available, in which mutation identification and monitoring is the key to selecting the right therapeutic approach.

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 4 fluorophores.

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) EGFR ex19	X	X					
EGFR del19 (between I744 and E749)		X					
EGFR del19 (between K754 and L760)	X						
EGFR L858R			X			X	
KRAS G12C					X	X	
KRAS G12D			X		X		
NRAS Q61R			X	X			
BRAF V600E				X	X		
IDH1 R132H				X		X	

Components

Pan-Cancer 1 (EGFR, KRAS, NRAS, BRAF, IDH1) 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
Pan-Cancer 1 Crystal Digital PCR® Assay	R51041	10X	Detects mutations in multiple genes EGFR, KRAS, NRAS, BRAF, IDH1
Pan-Cancer 1 Positive Control	R51041.PC0	10X	Contains: hgDNA, synthetic mutants EGFR E746-A750del, EGFR T751_I759>D, EGFR L858R, KRAS G12C, KRAS G12D, NRAS Q61R, BRAF V600E and IDH1 R132H

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 60 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 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_6C_Pan-Cancer1_R51041.ncx (6-color naica® system)
- NioProtocol_6C-60X-60°C-60s.nioprotocol (Nio® Digital PCR)
- NioAssay_6C_Pan-Cancer1_R51041.nioassay (Nio® Digital PCR)

Image Analysis

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

- CompensationMatrix_Prism6_Pan-Cancer1_R51041.ncm (6-color naica® system)
- CompensationMatrix_Nio_Pan-Cancer1_R51041 (Nio® Digital PCR)
- AnalysisConfiguration_Pan-Cancer1_R51041.nca (all systems)

Consumables Required but Not Provided

- Ruby Chip (C16011)
- naica® PCR MIX 10X (R10106)
- Crystal 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

Set thresholds for separating positive and negative populations on the 1D plots. To optimize the analysis, the thresholds should be set at approximately equal distance from the positive and negative clusters. Examples of results obtained on the Nio+ system are given below.

Remark: The threshold can be adjusted on each individual chamber to optimize its placement.

Wet lab testing was carried out using H_2O as a negative control and a positive control consisting of hgDNA and synthetic mutant target DNAs. Synthetic mutant target DNAs were also tested individually (EGFR E746-A750del, EGFR T751_I759>D, EGFR L858R, KRAS G12C, KRAS G12D, NRAS Q61R, BRAF V600E and IDH1 R132H).

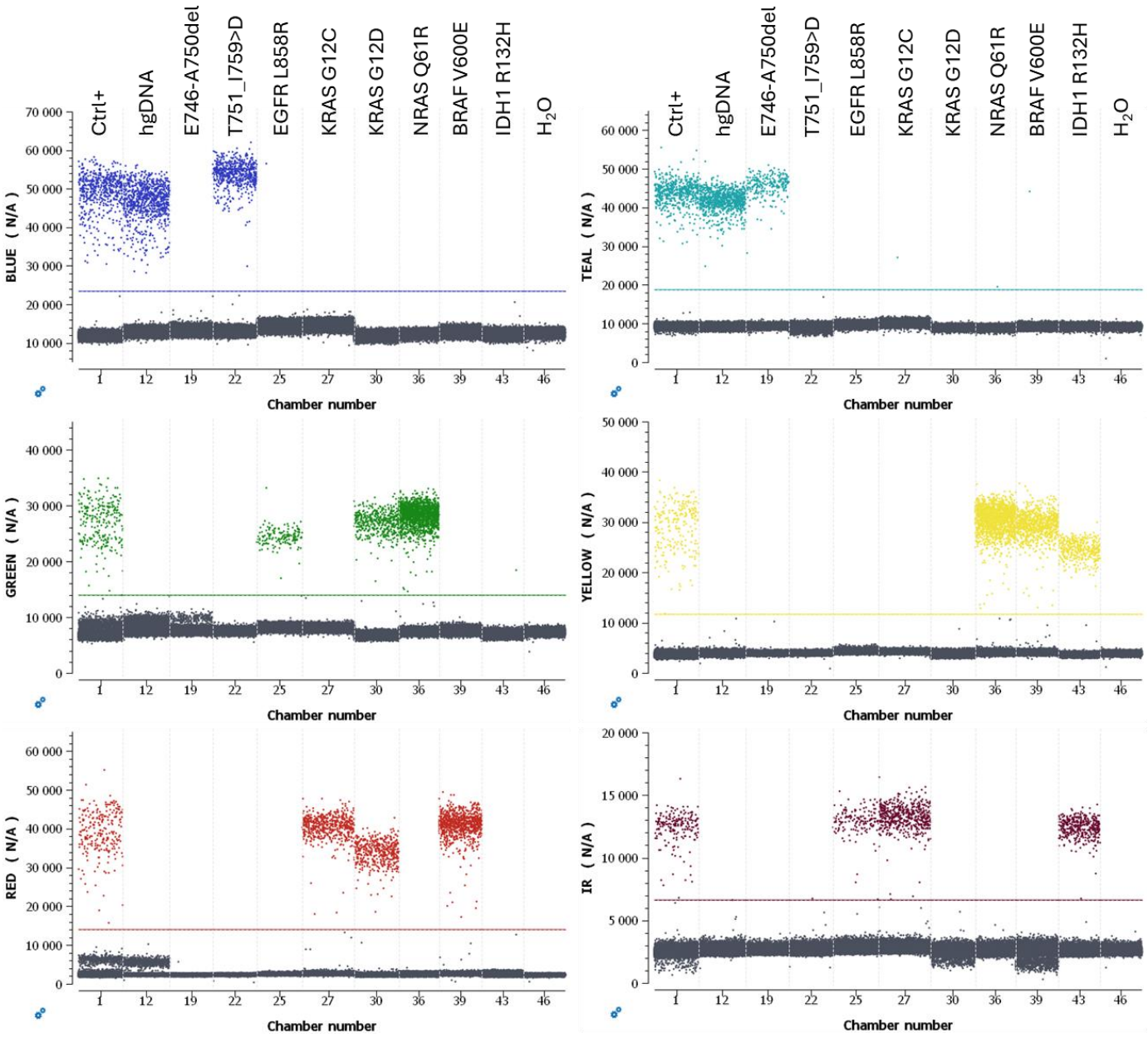

Figure 1: 1D plots obtained during wet lab testing on the Nio®+. *The thresholds should be set above negative cluster.*

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