

# 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
<b>EGFR deletion Exon19, EGFR L858R</b> <b>KRAS G12C, KRAS G12D</b> <b>NRAS Q61R</b> <b>BRAF V600E</b> <b>IDH1 R132H</b>	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 upon demand to Stilla's Technical Support team at [support-stilla@bio-rad.com](mailto:support-stilla@bio-rad.com).

Component Name	Reference	Concentration	Description
<b>Pan-Cancer 1 Crystal Digital PCR® Assay</b>	R51041	10X	Detects mutations in multiple genes EGFR, KRAS, NRAS, BRAF, IDH1
<b>Pan-Cancer 1 Positive Control</b>	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
<b>Step 1</b>	Partition for Ruby Chip	-
<b>Step 2</b>	Temperature 95°C for 180 seconds	1°C/sec
<b>Step 3</b>	Begin Loop for 60 Iterations	-
<b>Step 3.1</b>	Temperature 95°C for 15 seconds	1°C/sec
<b>Step 3.2</b>	Temperature 58°C for 60 seconds	1°C/sec
<b>Step 4</b>	Release for Ruby Chip	-

### On the Nio Digital PCR:

Step		Ramp rate
<b>Step 1</b>	Partition for Ruby Chip	-
<b>Step 2</b>	Temperature 95°C for 180 seconds	1°C/sec
<b>Step 3</b>	Begin Loop for 60 Iterations	-
<b>Step 3.1</b>	Temperature 95°C for 15 seconds	2°C/sec
<b>Step 3.2</b>	Temperature 60°C for 60 seconds	2°C/sec
<b>Step 4</b>	Temperature 58°C for 300 seconds	1°C/sec
<b>Step 5</b>	Release for Ruby Chip	-

## Data Acquisition

Download Nio dedicated technical files from [bio-rad.com](http://bio-rad.com).

- NioProtocol\_6C-60X-60°C-60s+58°C300s.nioprotocol (Nio Digital PCR)
- NioAssay\_6C\_Pan-Cancer1\_R51041.nioassay (Nio Digital PCR)

Download naica dedicated technical files from [bio-rad.com](http://bio-rad.com).

- ScanningTemplate\_Prism6\_6C\_Pan-Cancer1\_R51041.ncx (6-color naica system)

## Data 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 <span style="color: green;">●</span>	10x	1x	0.60
naica® PCR MIX Buffer B <span style="color: red;">●</span>	100%	4%	0.24
Crystal Digital PCR® Assay <span style="color: orange;">●</span>	10x	1x	0.60
Crystal Universal Reporter Tube A <span style="color: yellow;">●</span>	40x	1x	0.15
Crystal Universal Reporter Tube B <span style="color: brown;">●</span>	40x	1x	0.15
Nuclease-free water	NA	NA	Variable
Template DNA	<b>NA</b>	<b>NA</b>	<b>Variable</b>
<i>or Positive Control</i> <span style="color: grey;">○</span>	10x	1x	0.60
<b>Total reaction volume (µL)</b>			<b>6.0</b>

## DNA Digestion

DNA samples with ≥10 kb average length (e.g., genomic DNA) could be fragmented by restriction digestion before partitioning to ensure even distribution of the DNA template during partitioning. Restriction digestion is not required for highly fragmented DNA (e.g., FFPE DNA or circulating DNA). This step could improve assay performance and should be tested utilizing desired samples.

Care must be taken to use restriction enzymes that do not cut within the amplified sequence or the Crystal Flex Probes.

For a list of restriction enzymes compatible with a given Crystal Digital PCR assay, contact our Technical Support team ([support-stilla@bio-rad.com](mailto:support-stilla@bio-rad.com)).

## Loading Amount

For optimal performance, it is recommended not to exceed a chamber concentration (DNA concentration in the reaction mix) of 1,000 copies/ $\mu$ L. The performance of the assay at higher concentrations is not guaranteed and must be validated by the user.

## 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<sub>2</sub>O 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).

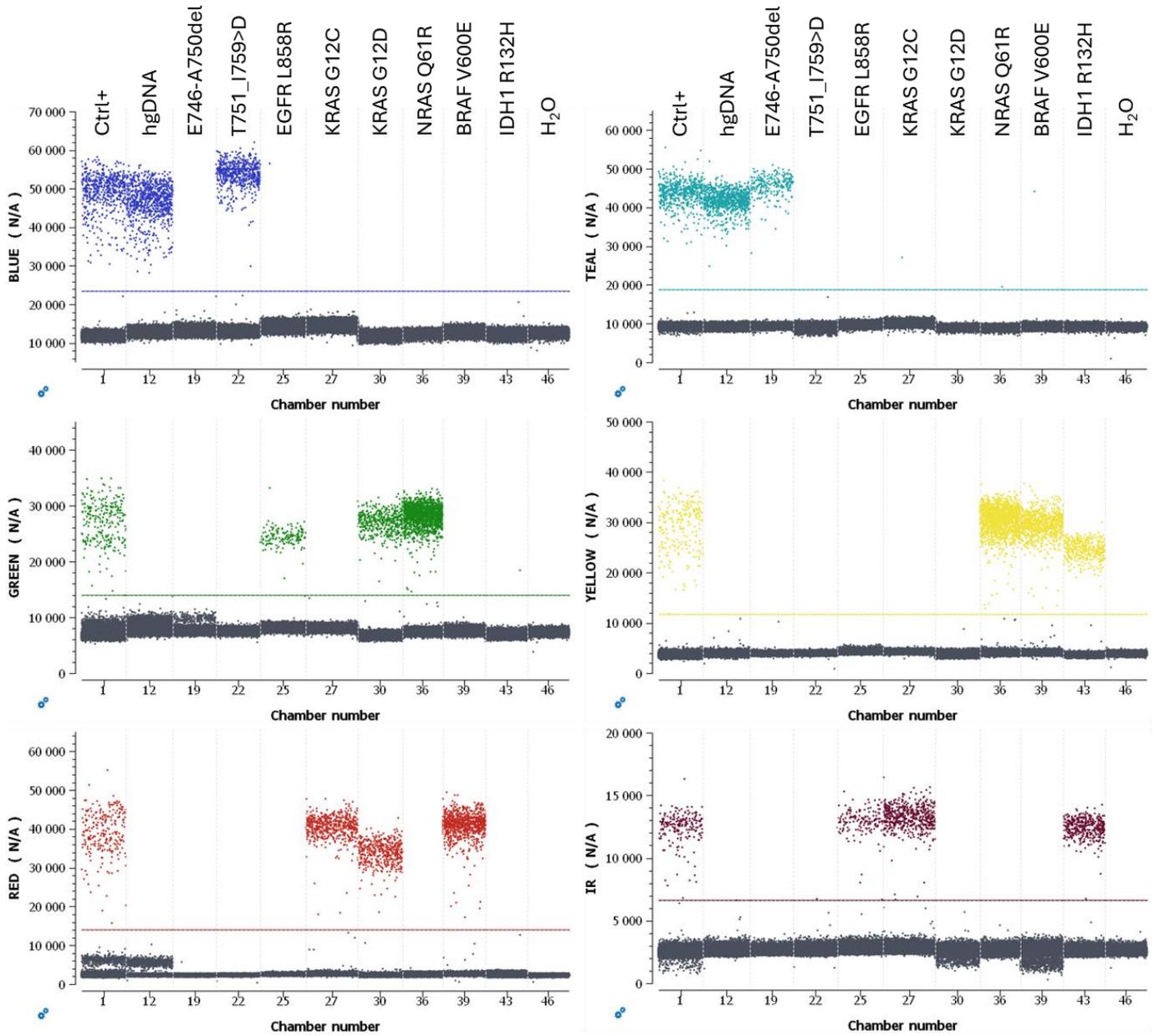


Figure 1: 1D plots obtained during wet lab testing on the Nio®+. The thresholds should be set above negative cluster.

## Calculation of Mutant Allelic Fraction (MAF)

The Mutant Allelic Fraction of each target can be calculated as follows:

$$\text{MAF EGFR Deletion ex19 (\%)} = \frac{[\text{EGFR Del19 754-759}] \text{ or } [\text{EGFR Del19 744-749}]}{[\text{EGFR Del19 754-759}] + [\text{EGFR Del19 744-749}] + [\text{EGFR ex19WT}]} * 100$$

- [EGFR Del19 754-759] = measured EGFR Del19 754-759 concentration
- [EGFR Del19 744-749] = measured EGFR Del19 744-749 concentration
- [EGFR ex19WT] = measured EGFR ex19WT concentration

*Remark:* adapt the formula according to detected EGFR deletion ex19 (deletion between I744 and E749 or deletion between K754 and L760).

For other mutations (EGFR L858R, BRAF V600E, KRAS G12C, KRAS G12D, NRAS Q61R, IDH1 R132H), proceed as follows:

$$\text{MAF MUT (\%)} = \frac{[\text{MUT}]}{[\text{EGFR Del19 754-759}] + [\text{EGFR Del19 744-749}] + [\text{EGFR ex19WT}]} * 100$$

- [MUT] = measured concentration of one mutation, such as BRAF V600E for example
- [EGFR Del19 754-759] = measured EGFR Del19 754-759 concentration
- [EGFR Del19 744-749] = measured EGFR Del19 744-749 concentration
- [EGFR ex19WT] = measured EGFR ex19WT concentration

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