Crystal Digital PCR® Assay

Information Sheet

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

Product Name

PIK3CA (Ref, H1047L, E542K, E545K, H1047R, N345K) Crystal Digital PCR® Assay (R51029)

Description

Detected Targets

Targets	Sample Type	Detection Channels	Multiplex
PIK3CA Ref, H1047L, E542K, E545K, H1047R, N345K	DNA	Blue/Teal/Green/ Yellow/Red/Infra-Red	6

The PIK3CA (Exon 10 reference, H1047L, E542K, E545K, H1047R, N345K) Crystal Digital PCR® Assay is a 10X assay designed to detect and quantify principal mutations in exons 5, 10 and 21 of the PIK3CA gene using the Ruby Chip. PIK3CA is essential for regulating multiple cellular processes through the PI3K/AKT/mTOR signaling pathway including cell growth, proliferation, survival, and metabolism.

Assay Configuration

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

Targets	Exon	Base changes	Blue	Teal	Green	Yellow	Red	Infra-Red	Long-Shift
PIK3CA exon 10 reference	10	N/A	X						
PIK3CA H1047L	21	c.3140A>T		X					
PIK3CA E542K	10	c.1624G>A	X		X				
PIK3CA E545K	10	c.1633G>A	X			X			
PIK3CA H1047R	21	c.3140A>G					X		
PIK3CA N345K	5	c.1035T>A						X	

Components

PIK3CA (Ref, H1047L, E542K, E545K, H1047R, N345K) 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
PIK3CA (Exon 10 reference, H1047L, E542K, E545K, H1047R, N345K) Crystal Digital PCR® Assay	R51029	10X	Detects 5 mutations in exons 5, 10, and 21 of the PIK3CA gene
PIK3CA Positive Control	R51035.PC0	10X	Contains: hgDNA and synthetic PIK3CA mutants (H1047R, E545K, E542K, N345K, H1047L, E726K, C420R, E453K, Q546R, G118D, E545A)

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_Prism6_PIK3CA_R51029.ncx (6-color naica® system)
- NioProtocol_6C-60X-60°C-30s.nioprotocol (Nio™ Digital PCR)
- NioAssay_6C_PIK3CA_R51029.nioassay (Nio™ Digital PCR)

Image Analysis

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

- CompensationMatrix_Prism6_PIK3CA_R51029.ncm (6-color naica® system)
- CompensationMatrix_Nio_PIK3CA_R51029.ncm (Nio™ Digital PCR)
- AnalysisConfiguration_PIK3CA_R51029.nca (all systems)

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

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 for all the channels. Examples of results obtained on the Nio ™ + are given below.

Wet lab testing was carried out using genomic hgDNA as a negative control and a positive control consisting of hgDNA and 11 synthetic PIK3CA mutants (H1047R, E545K, E542K, N345K, H1047L, E726K, C420R, E453K, Q546R, G118D, E545A). Synthetic PIK3CA mutants were also tested individually (H1047L, E542K, E545K, H1047R, N345K).

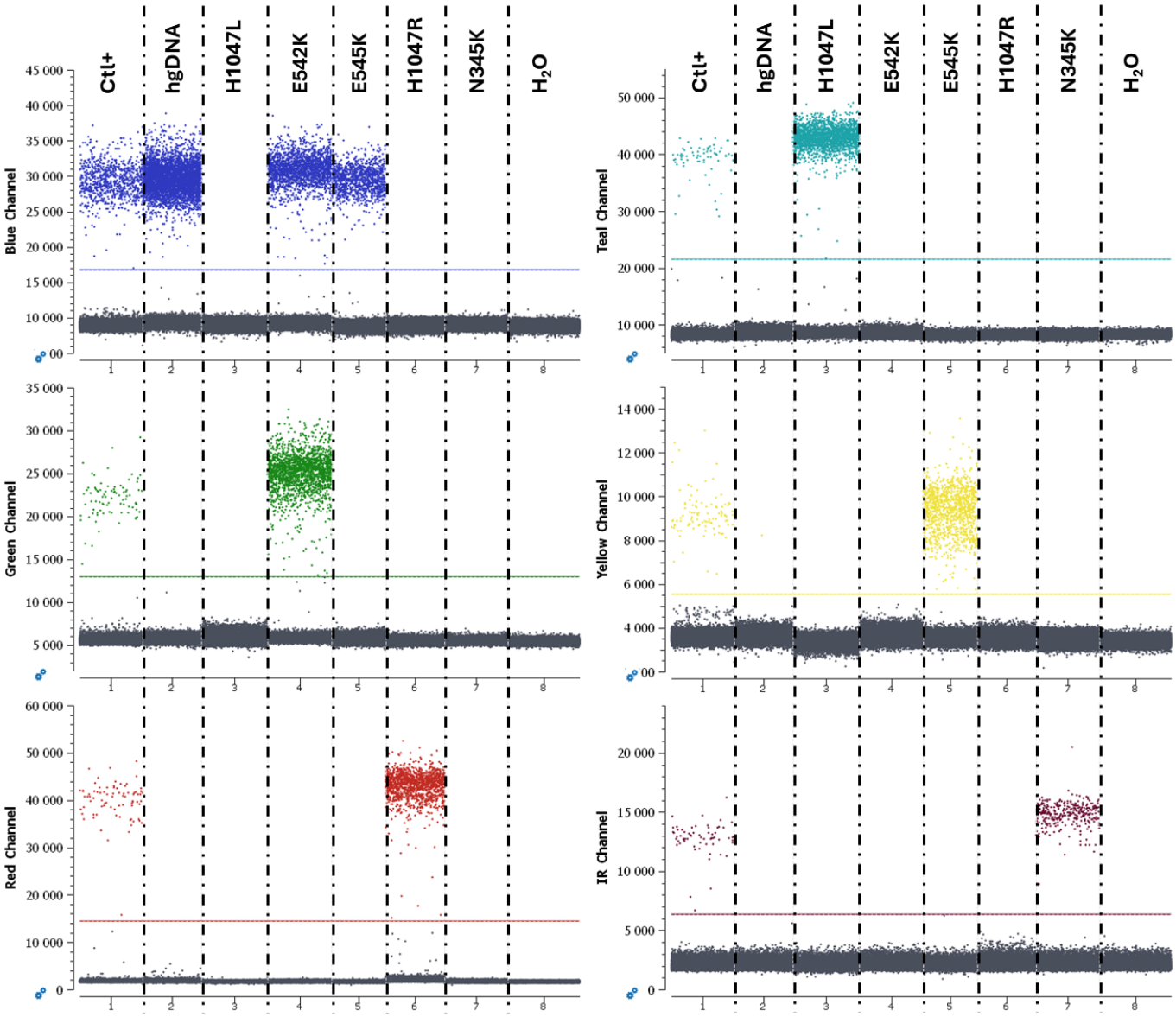

Figure 1: 1D plots obtained during wet lab testing on the Nio™+. *The thresholds are set at approximately equal distance from the positive and negative clusters.*

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