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 upon demand to Stilla’s Technical Support team at support-stilla@bio-rad.com.

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	R51029.PC0	10X	Contains: hgDNA and synthetic PIK3CA mutants (H1047R, E545K, E542K, N345K, H1047L)

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	-

Data Acquisition

Download Nio dedicated technical files from bio-rad.com.

- NioProtocol_6C-60X-60°C-30s+58°C300s.nioprotocol (Nio Digital PCR)
- NioAssay_6C_PIK3CA_R51029.nioassay (Nio Digital PCR)

Download naica dedicated technical files from bio-rad.com.

- ScanningTemplate_Prism6_PIK3CA_R51029.ncx (6-color naica system)

Data 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***

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).

Loading Amount

For optimal performance, it is recommended not to exceed a chamber concentration (DNA concentration in the reaction mix) of 1,000 copies/µL. 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 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).

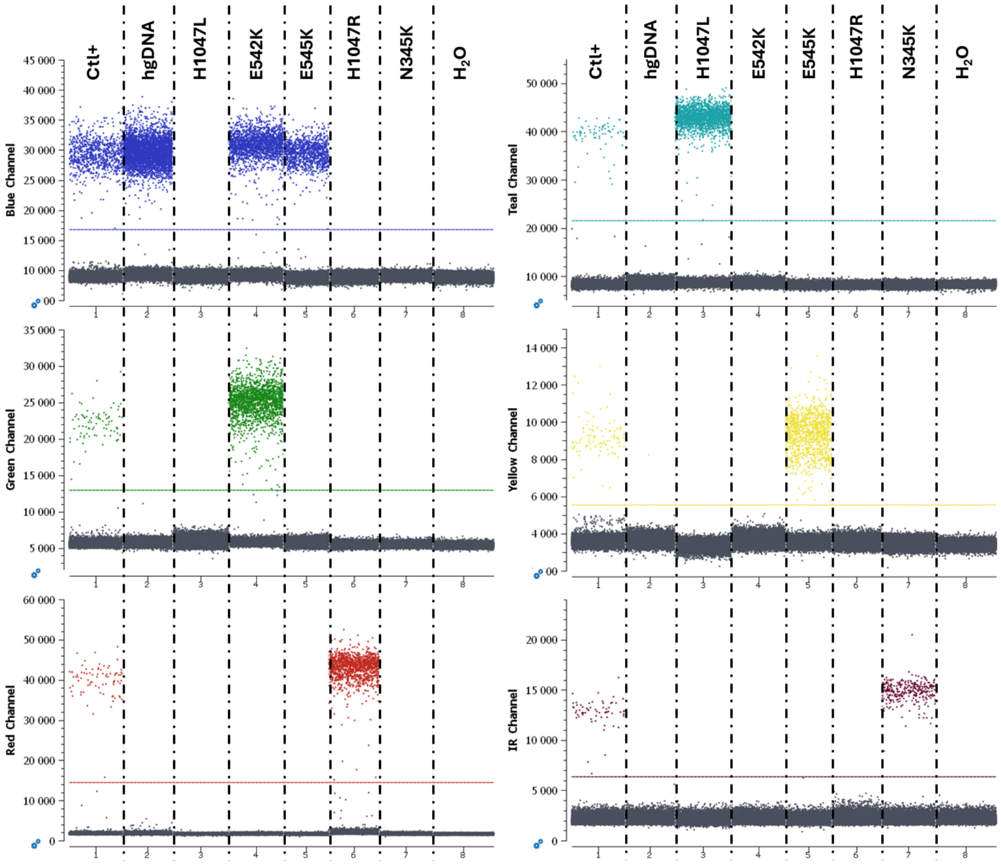
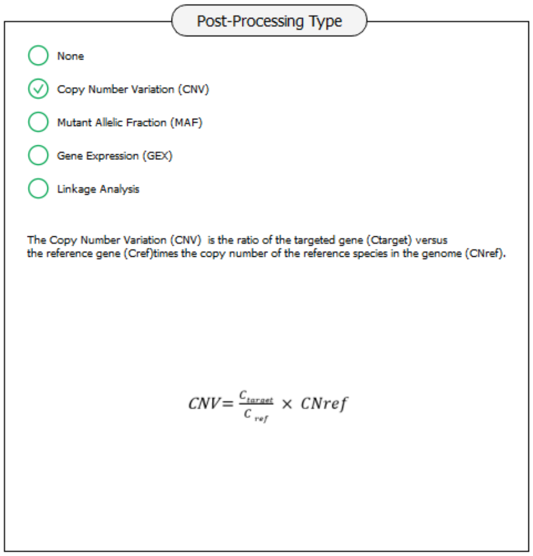


Figure 1: 1D plots obtained during wet lab testing on the Nio+. *The thresholds are set at approximately equal distance from the positive and negative clusters.*

Post-Processing (only available with NioAnalyzer software)

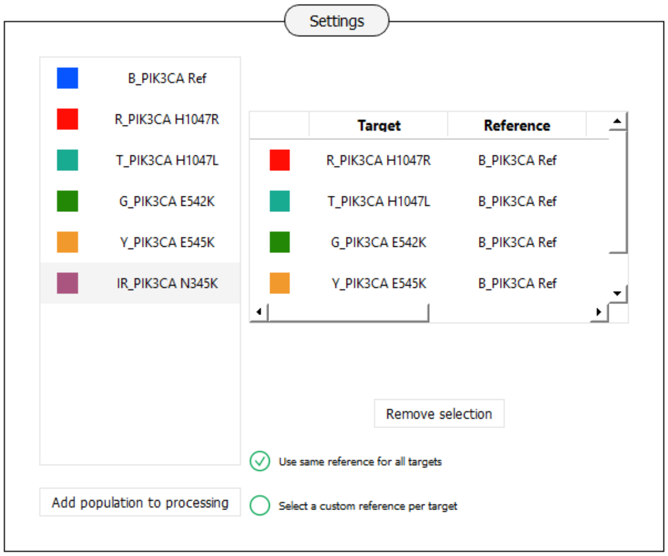
To perform a post-processing analysis of the results, click on “Setup” in the “POST PROCESSING” menu and select the appropriate analysis: **Copy Number Variation (CNV)**. Follow specific instructions for this assay:



- ○ None
- ☑ Copy Number Variation (CNV)
- ○ Mutant Allelic Fraction (MAF)
- ○ Gene Expression (GEX)
- ○ Linkage Analysis

The Copy Number Variation (CNV) is the ratio of the targeted gene (Ctarget) versus the reference gene (Cref)times the copy number of the reference species in the genome (CNref).

$$CNV = \frac{C_{target}}{C_{ref}} \times CNref$$



- 🟦 B_PIK3CA Ref
- 🟥 R_PIK3CA H1047R
- 🟩 T_PIK3CA H1047L
- 🟩 G_PIK3CA E542K
- 🟧 Y_PIK3CA E545K
- 🟪 IR_PIK3CA N345K

	Target	Reference
🟥	R_PIK3CA H1047R	B_PIK3CA Ref
🟩	T_PIK3CA H1047L	B_PIK3CA Ref
🟩	G_PIK3CA E542K	B_PIK3CA Ref
🟧	Y_PIK3CA E545K	B_PIK3CA Ref

- ☑ Use same reference for all targets
- ○ Select a custom reference per target

All populations should be added to processing, and “B_PIK3CA Ref” selected as reference.

Clicking on apply will launch the calculation. The values will be displayed in the “Results” tab.

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