Crystal Digital PCR® Assay

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

Product Name

c-KIT D816V Crystal Digital PCR Assay (R51010)

Description

Detected Targets

Targets	Sample Type	Detection Channels	Multiplex
c-KIT D816V	DNA	Blue/Green	2

The c-KIT D816V Crystal Digital PCR® Assay is a 10X assay designed to detect and quantify 1 mutation in the c-KIT gene using the Ruby Chip. c-KIT encodes for a receptor tyrosine kinase that plays a crucial role in cell signaling for processes including cell survival, proliferation, and differentiation.

Assay configuration

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

Targets	Base changes	Blue	Teal	Green	Yellow	Red	Infra-Red	Long-Shift
c-KIT reference	N/A	X						
c-KIT D816V	c2447 A>T	X		X				

c-KIT D816 WT DNA ——————————

c-KIT D816V DNA ——————————

Blue = Reference Flex Probe
Green = Mutant Flex Probe

Figure 1: Color assignment according to targets.

Components

The c-KIT D816V 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
c-KIT D816V Crystal Digital PCR® Assay	R51010	10X	Detects 1 mutation in the c-KIT gene
c-KIT Positive Control	R51010.PC0	10X	Contains: hgDNA and synthetic c-KIT D816V mutant

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 45 Iterations	-
Step 3.1	Temperature 95°C for 15 seconds	1°C/sec
Step 3.2	Temperature 62°C for 30 seconds	1°C/sec
Step 4	Temperature 55°C for 600 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 55°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_3C-60X-60°C-30s+55°C300s.nioprotocol (Nio Digital PCR)
- NioAssay_3C_cKIT_R51010.nioassay (Nio Digital PCR)

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

- ScanningTemplate_Prism3_cKIT_R51010.ncx (3-color naica system)
- ScanningTemplate_Prism6_cKIT_R51010.ncx (6-color naica system)

Data Analysis

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

- CompensationMatrix_Prism3_cKIT_R51010.ncm (3-color naica system)
- UniversalCompMatrix_3C_Prism6-Nio.ncm (6-color naica system, Nio Digital PCR)
- AnalysisConfiguration_cKIT_R51010.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*

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 blue and the green thresholds should be set at approximately equal distance from the positive and negative clusters. Examples of results obtained on the 3-color naica system are given below.

Wet lab testing was carried out using genomic hgDNA and H_2O as negative controls and a positive control consisting of hgDNA and synthetic c-KIT D816V mutant.
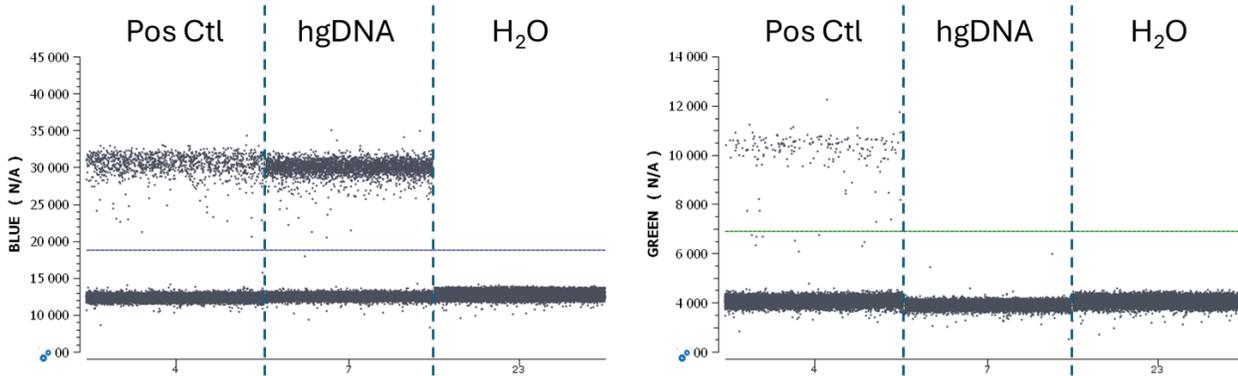
Figure 2: 1D plots obtained during wet lab testing on the 3-color naica® system. The blue and the green thresholds should be set at approximately equal distance from the positive and negative clusters.

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:

All populations should be added to processing, and "c-KIT reference" selected as reference.

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

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