Crystal Digital PCR® Assay

Information Sheet

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

Product Name

IDH1 (R132, R132H, R132C) Crystal Digital PCR® Assay (R51051)

Description

Detected Target

Targets	Sample Type	Detection Channels	Multiplex
IDH1 (R132, R132H, R132C)	DNA	Blue/Green/Red	3

The IDH1 (R132, R132H, R132C) Crystal Digital PCR® Assay is a 10X assay designed to detect and quantify 2 mutations in the Isocitrate Dehydrogenase 1 (IDH1) gene using the Ruby Chip. IDH1 encodes an enzyme that catalyzes the oxidative decarboxylation of isocitrate to α-ketoglutarate (α-KG). It is involved in energy metabolism (Krebs cycle). Mutations in IDH1 – particularly R132H – are found in several cancers, most notably in Gliomas and Acute myeloid leukemia (AML).

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
Wild-type (WT) IDH1	N/A	X						
IDH1 R132H	c.395G>A			X				
IDH1 R132C	c.394C>T					X		

Components

The IDH1 (R132, R132H, R132C) Crystal Digital PCR® Assay comprises two reagents: a pool of the assay specific primers and Crystal Flex Probes and Positive Control. Please refer to the lot specific Certificate of Conformity for characterized concentration, available for upon request from Stilla’s Technical Support team (support@stilla.fr).

Component Name	Reference	Concentration	Description
IDH1 (R132, R132H, R132C) Crystal Digital PCR® Assay	R51051	10X	Detects 2 mutations in the IDH1 gene
IDH1 (R132, R132H, R132C) Positive Control	R51051.PC0	10X	Contains: hgDNA, synthetic IDH1 R132C and R132H mutants

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 40 Iterations	-
Step 3.1	Temperature 95°C for 15 seconds	1°C/sec
Step 3.2	Temperature 60°C for 30 seconds	1°C/sec
Step 4	Temperature 58°C for 900 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 40 Iterations	-
Step 3.1	Temperature 95°C for 15 seconds	2°C/sec
Step 3.2	Temperature 62°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_3C-40X-62°C-30s+58°C300.nioprotocol (Nio Digital PCR)
- NioAssay_3C_IDH1_R51051.nioassay (Nio Digital PCR)

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

- ScanningTemplate_Prism3_ IDH1_R51051_v1.ncx (3-color naica system)
- ScanningTemplate_Prism6_ IDH1_R51051_v1.ncx (6-color naica system)

Data Analysis

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

- CompensationMatrix_Prism3_IDH1_R51051_V1.ncm (3-color naica system)
- CompensationMatrix_Prism6_ IDH1_R51051_V1.ncm (6-color naica system)
- CompensationMatrix_Nio_IDH1_R51051_v1.ncm (6-color naica system, Nio Digital PCR)
- AnalysisConfiguration_Nio_IDH1_R51051.nca (all systems)
- PlotConfiguration_Nio_IDH1_R51051

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. Examples of results obtained on the Nio+ system are given below.
To optimize the analysis, the blue, red and green thresholds should be set just below the positive clusters as shown in the example.

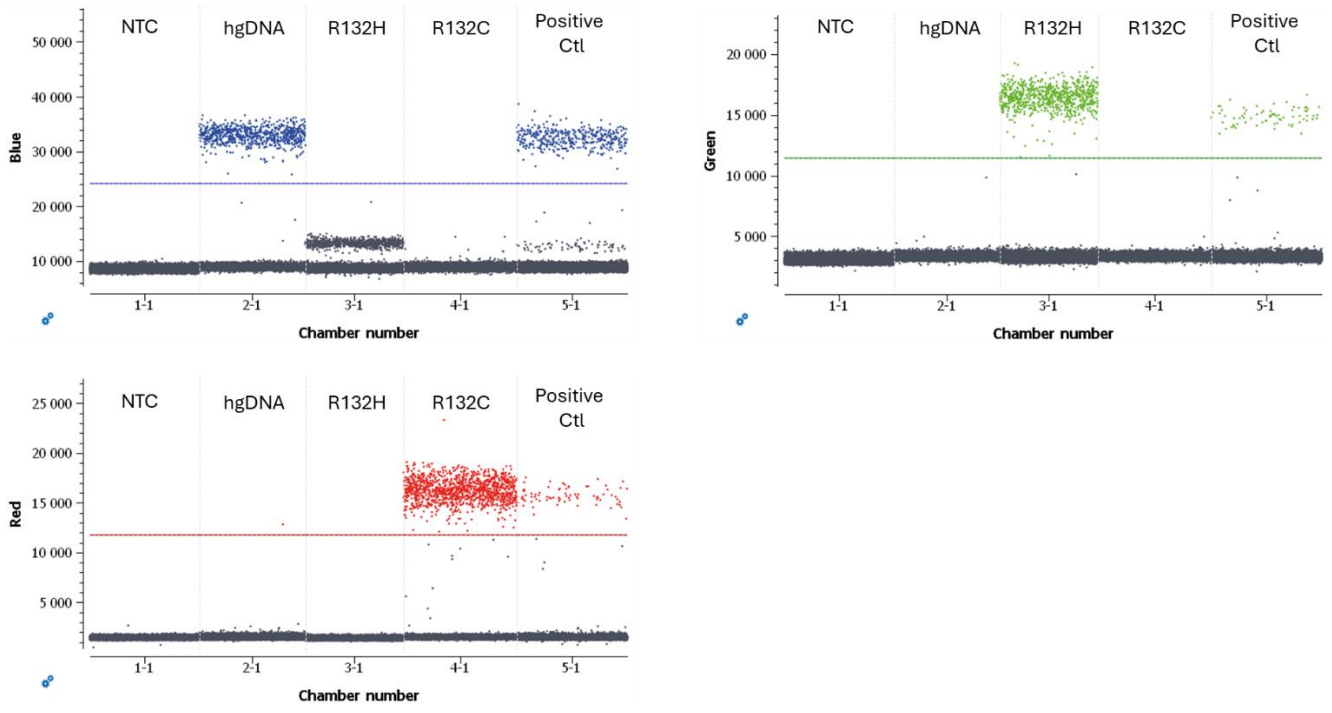
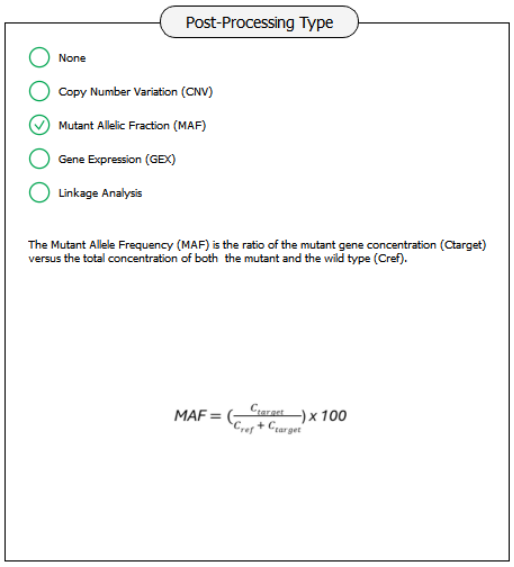


Figure 1: 1D plots obtained during wet lab testing on the Nio. *The blue, red and green thresholds are set just below the positive clusters.*

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: **Mutant Allelic Fraction (MAF)**. Follow specific instructions for this assay:



All populations should be added to processing, and “IDH1 R132 WT” selected as reference.

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

Stilla Technologies
F-94800 Villejuif, FRANCE