Crystal Digital PCR® Assay

Information Sheet

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

Product Name

EGFR rare (G719A/C/D/S, S768I, T790M, C797S, L861Q) Crystal Digital PCR® Assay (R51049)

Description

Detected Targets

Targets	Detection Channels	Multiplex
EGFR (ex18 ref, G719A, G719C, G719D, G719S, S768I, T790M, C797S, L861Q)	Blue / Teal / Green / Yellow / Red / IR	10

The EGFR rare (G719A/C/D/S, S768I, T790M, C797S, L861Q) Crystal Digital PCR® Assay is a 10X assay designed to detect and quantify 9 EGFR rare mutations using the Ruby Chip. EGFR encodes for the epidermal growth factor receptor, a protein that plays a crucial role in cell growth, proliferation, and differentiation.

Assay Configuration

This assay relies on the Color-Combination multiplexing strategy proprietary to Stilla Technologies, in which each target is detected, differentiated, and quantified by Crystal Digital PCR® using 2 fluorophores.

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

Target	Exon	Base change	Blue	Teal	Green	Yellow	Red	Infra-red	Long-Shift
Exon18 reference	18	n.a.	X						
G719A	18	c.2156G>C	X	X					
G719C	18	c.2155G>T	X			X			
G719D	18	c.2156G>A	X		X				
G719S	18	c.2155G>A	X					X	
S768I	20	c.2303G>T		X				X	
T790M	20	c.2369C>T					X	X	
C797S (T>A)	20	c.2389T>A				X		X	
C797S (G>C)	20	c.2390G>C			X	X			
L861Q	21	c.2582T>A			X		X		

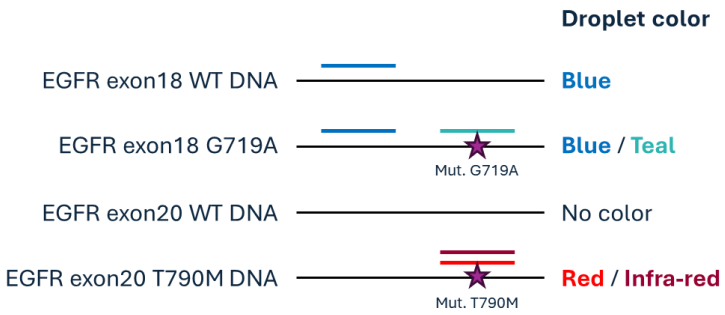


Figure 1: Example of color assignment according to targets.

Components

The assay comprises two reagents: a pool of the assay specific primers and Crystal Flex Probes and a pool of 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
EGFR rare Crystal Digital PCR® Assay	R51049	10X	Detection of the exon18 reference and 9 rare EGFR mutations
EGFR rare Positive Control	R51049.PC0	10X	Contains WT DNA and synthetic DNA of 9 EGFR mutations

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 60 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 60 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-60s+58°C300s.nioprotocol (Nio Digital PCR)
- NioAssay_6C_EGFR-rare_R51049.nioassay (Nio Digital PCR)

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

- ScanningTemplate_Prism6_6C_EGFR-rare_R51049_v1.ncx (6-color naica system)

Data Analysis

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

- CompensationMatrix_Prism6_EGFR-rare_R51049.ncm (6-color naica® system)
- CompensationMatrix_Nio_EGFR-rare_R51049.ncm (Nio® Digital PCR)
- AnalysisConfiguration_EGFR-rare_R51049.nca (all systems)

Consumables Required but Not Provided

- Ruby Chip (C16011)
- naica® PCR MIX 10X (R10106)
- Crystal Universal Reporters 7 (R42401 200 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 just below the positive cluster except for the Blue channel for which the threshold should be set at approximately equal distance from the positive and negative clusters. Examples of results obtained on the 6-color naica® system are given below.

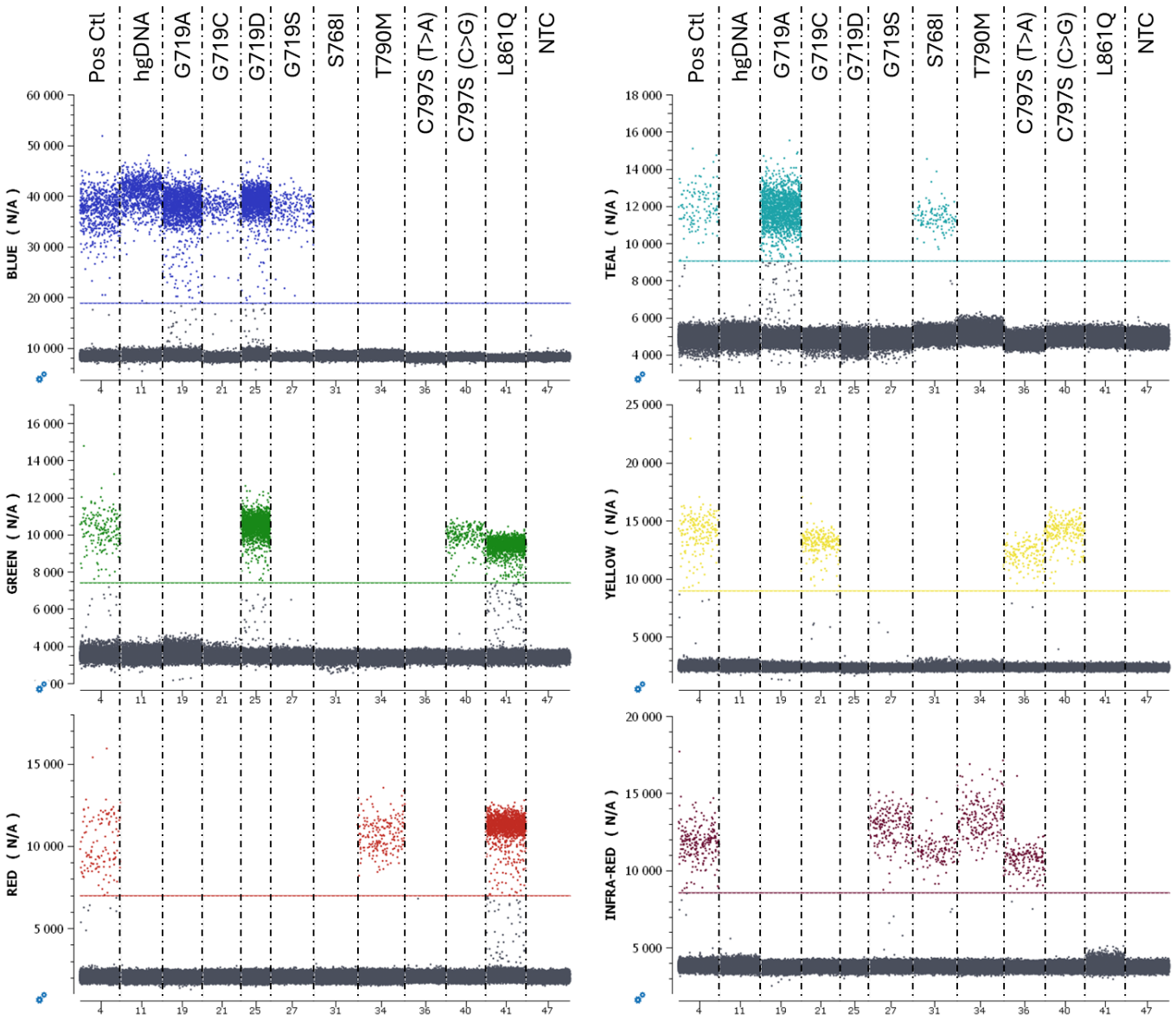
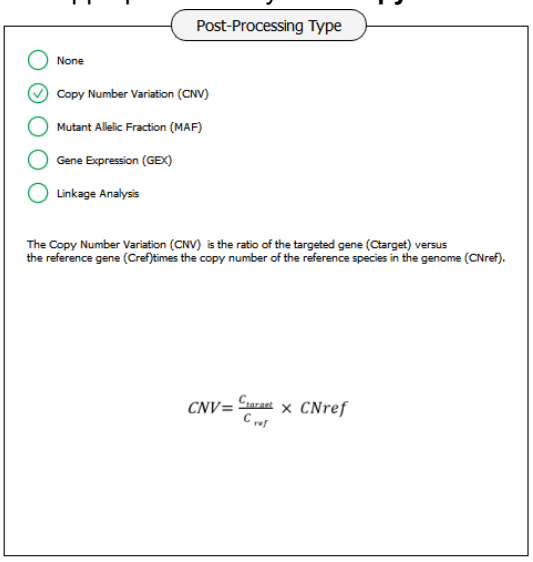
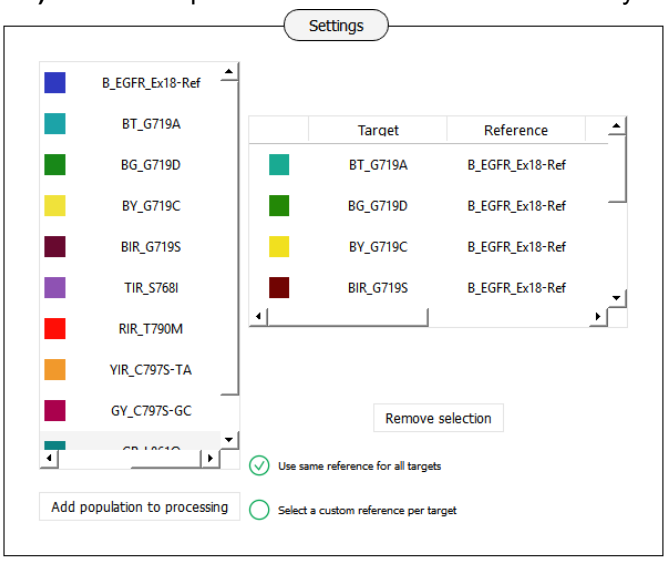


Figure 2: 1D plots obtained during testing on the 6-color Naica® system. *The thresholds should be set just below the positive cluster except for the Blue channel for which the threshold should be set at approximately equal distance from the positive and negative clusters*

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 “B_EGFR_Ex18-Ref” selected as reference.

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

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