Crystal Digital PCR® Assay

Information Sheet

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

Product Name

EGFR CNV (vIII, KDD) Crystal Digital PCR® Assay (R51050)

Description

Detected Targets

Targets	Detection Channels	Multiplex
HMBS, EGFR ref, EGFR vIII, EGFR KDD	Blue / Yellow / Red / IR	4

The EGFR CNV (vIII, KDD) Crystal Digital PCR® Assay is a 10X assay designed to detect and quantify EGFR duplication, EGFR variant III (deletion exons2-7) and EGFR Kinase Domain Duplication (exons 18-25 duplication) using the Ruby Chip. The copy number variation (CNV, i.e. duplication or deletion of gene or gene fragment) is given by the ratio of the estimated concentration of the target gene to the estimated concentration of the reference gene. EGFR encodes for the epidermal growth factor receptor, a protein that plays a crucial role in cell growth, proliferation, and differentiation.

Assay Configuration

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

Target	Gene position	Blue	Teal	Green	Yellow	Red	Infra-red	Long-Shift
HMBS	Intron 1	X						
EGFR ref	Intron 1					X		
EGFR vIII	Exon 3 / Intron 3						X	
EGFR KDD	Intron 22				X			

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 CNV Crystal Digital PCR® Assay	R51050	10X	Detection of the HMBS gene and EGFR gene at different positions
EGFR CNV Positive Control	R51050.PC0	10X	Contains WT DNA

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-CNV_R51050_v1 (Nio Digital PCR)

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

- ScanningTemplate_Prism6_6C_EGFR-CNV_R51050_v1.ncx (6-color naica system)

Data Analysis

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

- CompensationMatrix_Prism6_EGFR-CNV_R51050.ncm (6-color naica system)
- CompensationMatrix_Nio_EGFR-CNV_R51050.ncm (Nio Digital PCR)
- AnalysisConfiguration_EGFR-CNV_R51050.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

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.

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 just above the negative clusters. Examples of results obtained on the 6-color naica® system are given below.

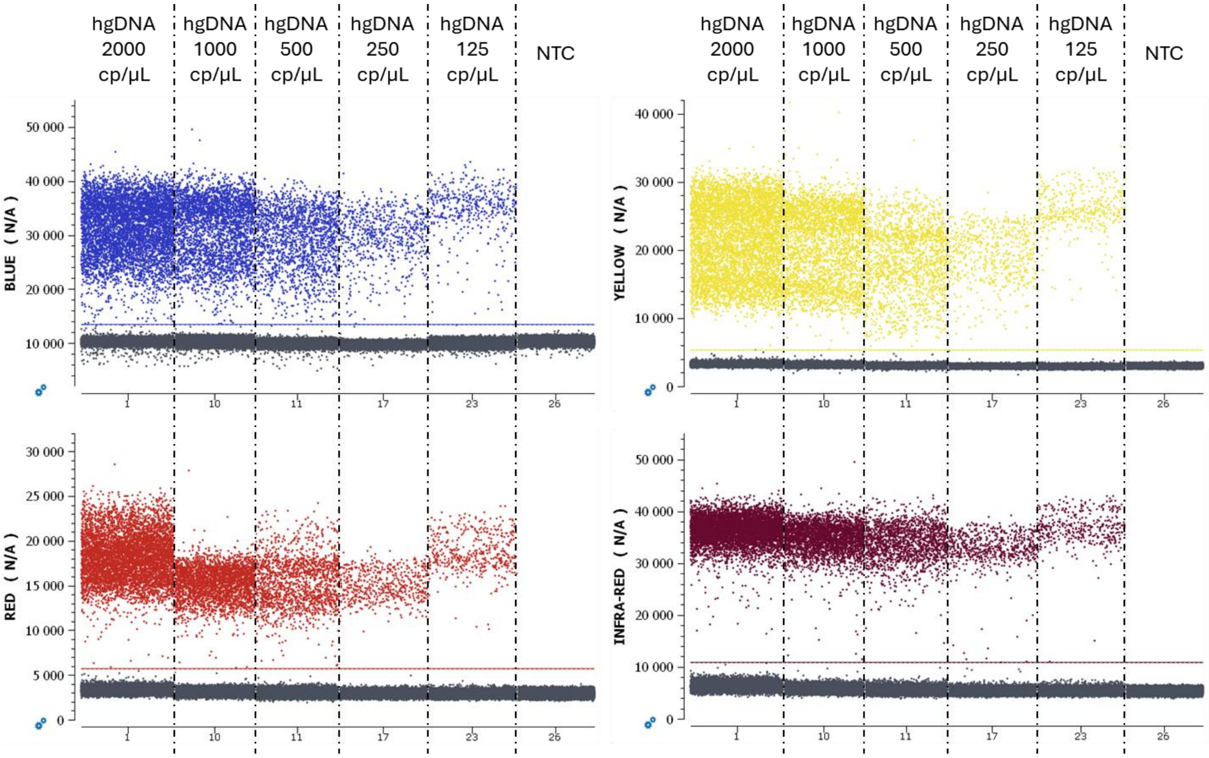
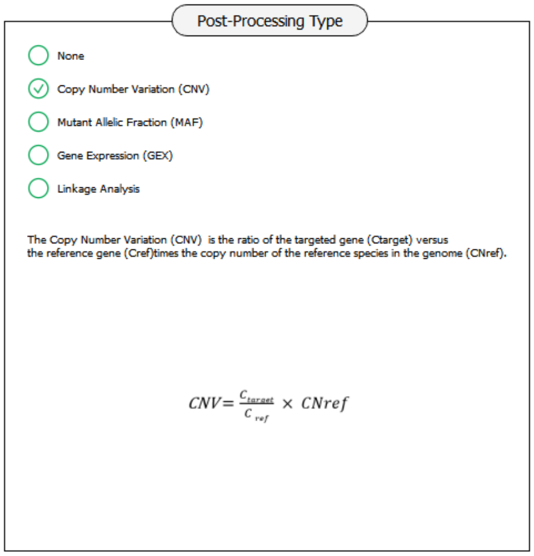


Figure 1: 1D plots obtained during testing on the 6-color Naica® system. *The thresholds should be set just above the negative clusters.*

Post-Processing (only available with NioAnalyzer software)

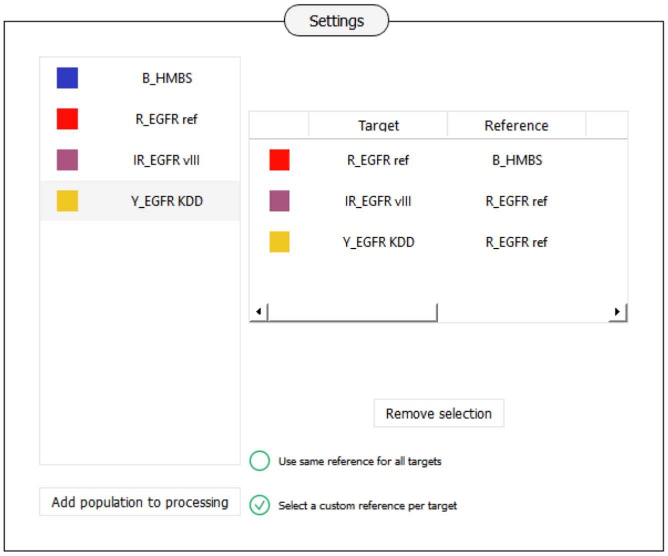
To perform a post-processing analysis of the results, click on “Setup” in the “POST PROCESSING” menu and select the appropriate analysis: **Copy Number Variation (CNV)**. Follow specific instructions for this assay:



○ 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_HMBS
🟥 R_EGFR ref
🟪 IR_EGFR vIII
🟨 Y_EGFR KDD

	Target	Reference
🟥	R_EGFR ref	B_HMBS
🟪	IR_EGFR vIII	R_EGFR ref
🟨	Y_EGFR KDD	R_EGFR ref

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

Add all populations to processing, and select specific reference for each:

- “B_HMBS” selected as reference for “R_EGFR ref” population,
- “R_EGFR ref” selected as reference for “IR_EGFR vIII” population,
- “R_EGFR ref" selected as reference for ‘Y_EGFR KDD” population.

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