Crystal Digital PCR® Assay

Information Sheet

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

Product Name

ESR1 (17 mutations) Crystal Digital PCR® Assay (R51011)

Description

Detected Targets

Targets	Sample Type	Detection Channels	Multiplex
ESR1 17 mutations	DNA	Blue/Teal/Green/ Yellow/Red/Infra-Red/LSSD	18

ESR1 (17 mutations) Crystal Digital PCR® Assay is a 10X assay designed to detect and quantify 17 mutations in the ESR1 gene using the Ruby Chip. ESR1 is pivotal in mediating resistance to endocrine therapy in metastatic hormone-positive breast cancer.

Multiplexing Strategy: Color-Combination

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(s) are used for each target in the assay:

Target	Exon	Base changes	Blue	Teal	Green	Yellow	Red	Infra-Red	Long-Shift
ESR1 exon 8 reference	8	N/A							X
E380Q	5	c.1138G>C		X		X			
V422del	6	c.1265_1267del	X					X	
S463P	7	c.1387T>C		X			X		
D538G	8	c.1613A>G			X	X			X
D538N	8	c.1612G>A	X				X		X
L536H	8	c.1607T>A				X		X	X
L536P	8	c.1607T>C				X	X		X
L536Q_delinsAG	8	c.1607_1608delinsAG	X	X					X
L536R	8	c.1607T>G		X	X				X
Y537C	8	c.1610A>G					X	X	X
Y537D	8	c.1609T>G		X				X	X
Y537H	8	c.1609T>C	X			X			X
Y537N	8	c.1609T>A			X			X	X
Y537N_delinsTA	8	c.1608_1609delinsTA	X		X				X
Y537S	8	c.1610A>C			X		X		X
Y537S_delinsAG	8	c.1609_1610delinsAG					X		X
Y537S_delinsCA	8	c.1610_1611delinsCA					X		X

Remark: ESR1_Y537S_delinsAG and ESR1_ Y537S_delinsCA are co-detected in the Red channel and cannot be differentiated.

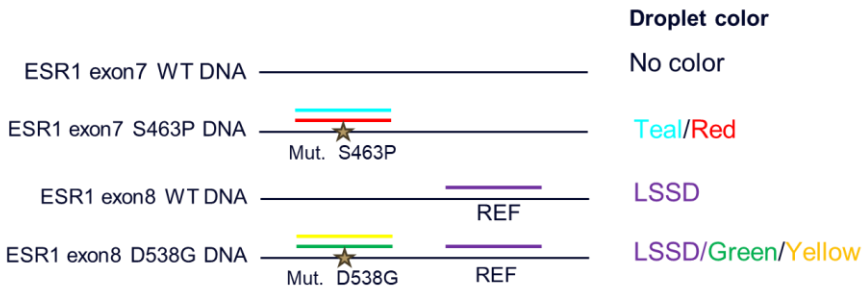


Figure 1: Example of color assignment according to targets. *The ESR1 Exon 8 reference is detected on the Long-Shift channel (LSSD) while mutations are specifically detected in two colors.*

Components

ESR1 (17 mutations) 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
ESR1 (17mutations) Crystal Digital PCR® Assay	R51011	10X	Detects 17 mutations in the ESR1 gene
ESR1 (17 mutations) Positive Control	R51011.PC0	10X	Contains: hgDNA + synthetic mutant sequences (17 mutations)

Thermocycling Programs

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 62°C for 45 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_7C-60X-62°C-45s+58°C300s.nioprotocol (Nio Digital PCR)
- NioAssay_7C_ESR1_51011.nioassay (Nio Digital PCR)

Data Analysis

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

- CompensationMatrix_Nio_ESR1_51011.ncm (Nio Digital PCR)
- AnalysisConfiguration_ESR1_51011.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 except for the Long Shift channel, for which the threshold should be set just above the negative group. Examples of results obtained on the Nio+ system are given below.

Wet lab testing was carried out using human genomic DNA (hgDNA) and H_2O negative controls and a positive control consisting of hgDNA and the 17 synthetic mutant target sequences. Synthetic mutant target sequences were also tested individually.

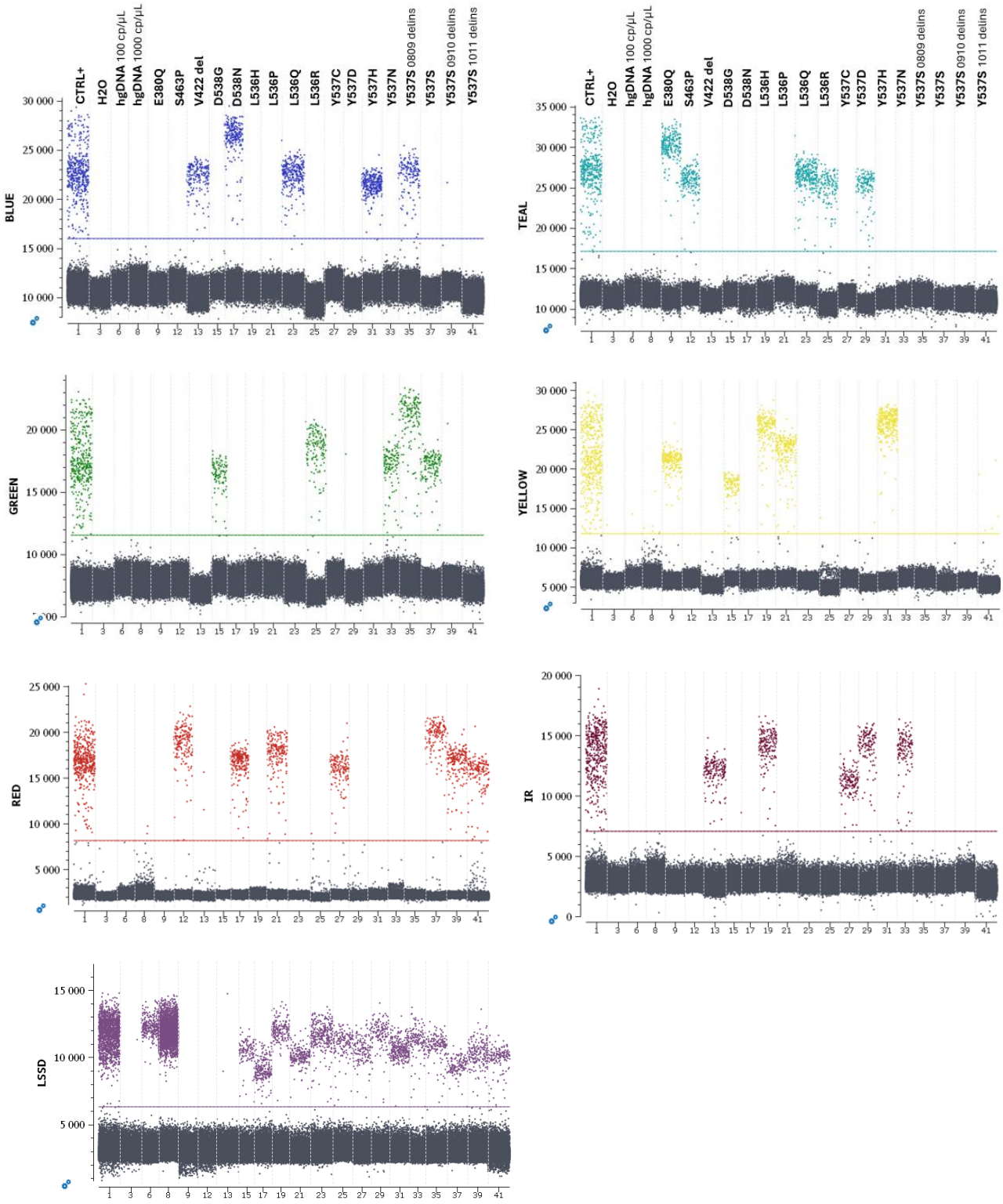


Figure 2: 1D plots obtained during wet lab testing on the Nio+. *The thresholds are set, based on positive control, at approximately equal distance from the positive and negative clusters.*

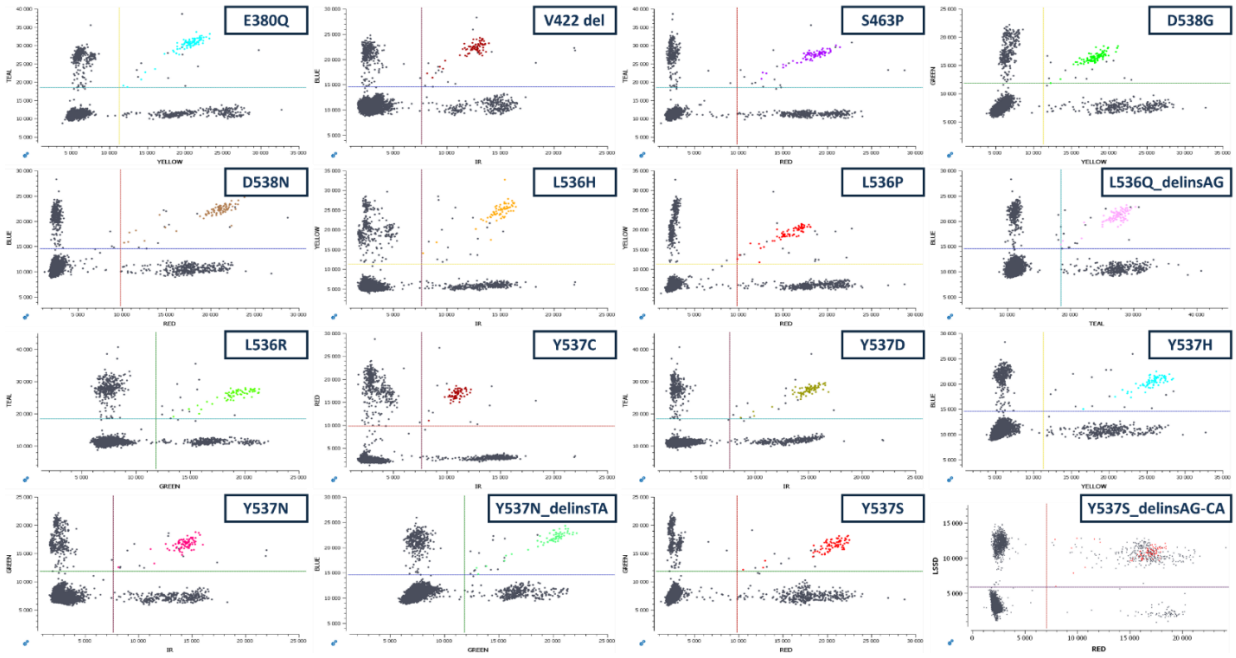
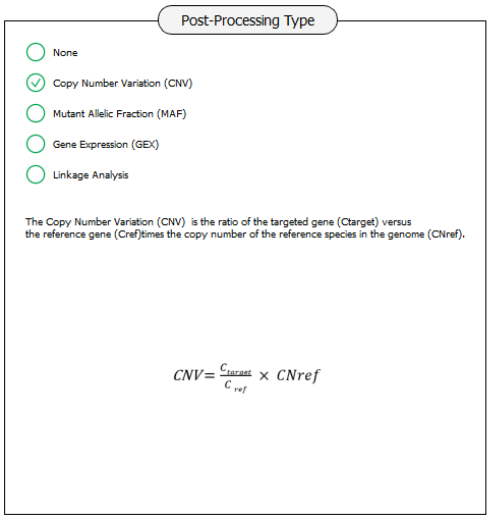
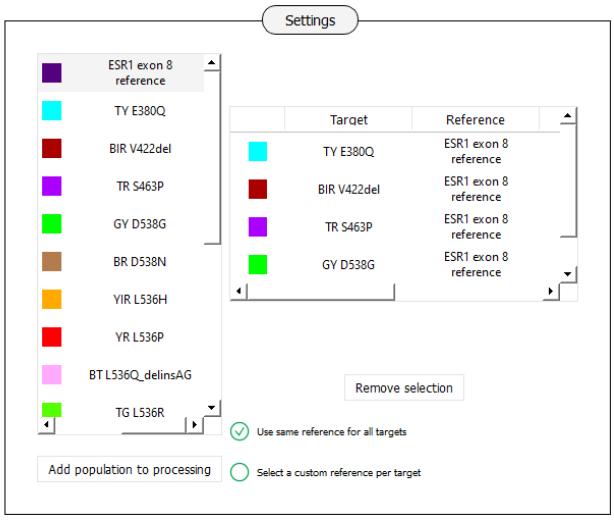


Figure 3: 2D plots obtained with the positive control during wet lab testing on the Nio+. *Each ESR1 mutation can be visualized as a double-positive population.*

Post-Processing

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 “ESR1 exon 8 reference” selected as reference.

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

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