Crystal Digital PCR® Assay

Information Sheet

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

Product Name

HPV (ALB, HPV16/18/31/33/35/39/45/51/52/56/58) Crystal Digital PCR® Assay (R52000)

Description

Detected Targets

Targets	Sample Type	Detection Channels	Multiplex
HPV (ALB, HPV16/18/31/33/35/39/45/51/52/56/58)	DNA	Blue/Teal/Green/Yellow/Red/Infra-Red	12

The HPV (ALB, HPV16/18/31/33/35/39/45/51/52/56/58) Crystal Digital PCR® Assay is a 10X assay designed to detect and quantify 11 high risk HPV genotypes for cervical cancer plus a reference gene for human DNA quantification using the Ruby Chip.

Multiplexing Strategy: Color-Combination

This assay relies on the Color-Combination multiplexing strategy proprietary to Stilla Technologies, in which targets are 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:

Targets	Blue	Teal	Green	Yellow	Red	Infra-Red	Long-Shift
ALB (human REF gene)	X						
HPV16		X			X		
HPV18			X	X			
HPV31		X	X				
HPV33	X				X		
HPV35		X				X	
HPV39		X		X			
HPV45			X		X		
HPV51			X			X	
HPV52				X		X	
HPV56					X	X	
HPV58	X			X			

Components

HPV (ALB, HPV16/18/31/33/35/39/45/51/52/56/58) 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
HPV (ALB, HPV16/18/31/33/35/39/45/51/52/56/58) Crystal Digital PCR® Assay	R52000	10X	Detects 11 high risk HPV genotypes for cervical cancer plus a reference gene for human DNA.
HPV Positive Control	R52000.PC0	10X	Contains: hgDNA, Synthetic HPV (16/18/31/33/35/39/45/51/52/56/58)

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 60°C for 60 seconds	1°C/sec
Step 4	Temperature 58°C for 300 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 62°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-62°C-60s+58°C300s.nioprotocol (Nio Digital PCR)
- NioAssay_6C_HPV12_R52000.nioassay (Nio Digital PCR)

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

- ScanningTemplate_Prism6_HPV12_R52000.ncx (6-color naica system)

Data Analysis

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

- CompensationMatrix_Prism6_HPV12_R52000.ncm (6-color naica system)
- CompensationMatrix_Nio_HPV12_R52000.ncm (Nio Digital PCR)
- AnalysisConfiguration_HPV12_R52000.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.

The thresholds should be set at approximately equal distance from the positive and negative clusters. Examples of results obtained on the Prism6 system are given below.

Wet lab testing was carried out using human genomic DNA (hgDNA) as a negative control and a positive control (Ctl+) consisting of hgDNA and 11 synthetic HPV subtypes (HPV16/18/31/33/35/39/45/51/52/56/58). Each HPV DNA was also tested individually to confirm the specificity of the assay (Figure 1).

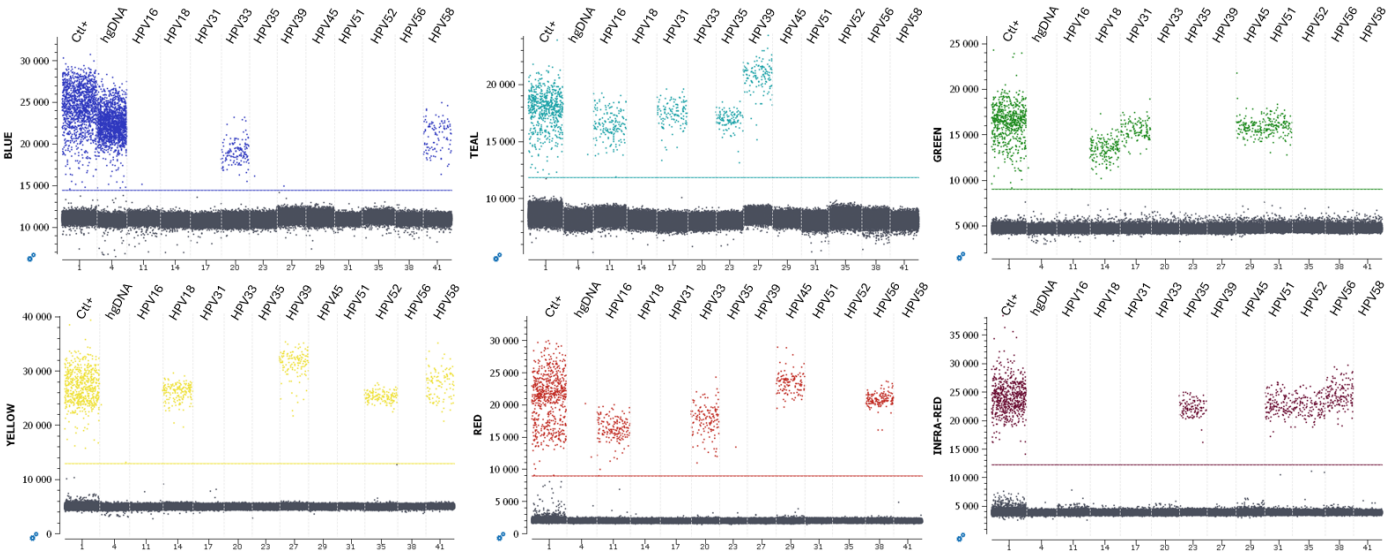

Figure 1: 1D plots obtained during wet lab testing on the Prism6. *The blue threshold is set just above the negative cluster, while the five other thresholds are set at approximately equal distance from the positive and negative clusters.*

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