Crystal Digital PCR® Assay

Information Sheet

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

Product Name

Kanamycin Resistance Gene Crystal Digital PCR® Assay (R53000)

Description

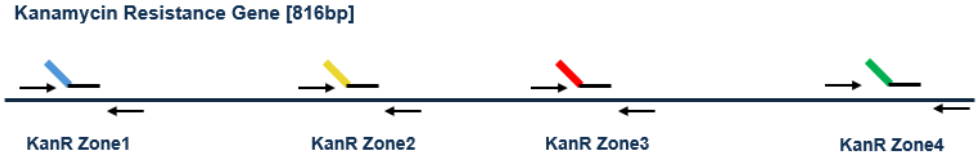
Detected Targets

Targets	Sample Type	Detection Channels	Multiplex
Kanamycin Resistance Gene (KanR)	DNA	Blue/Green/Yellow/Red	4

Kanamycin Resistance Gene Crystal Digital PCR® Assay is a 10X assay designed to detect and quantify four sequences of the KanR gene covering the whole gene, using the Ruby Chip. Detecting KanR gene in cell and gene therapy products is essential to meet regulatory standards, ensuring patient safety by minimizing risks of antibiotic resistance and immune responses.

Assay Configuration

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



Gene	Blue	Teal	Green	Yellow	Red	Infra-Red	Long-Shift
KanR zone1	X						
KanR zone2				X			
KanR zone3					X		
KanR zone4			X				

Components

Kanamycin Resistance Gene 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 concentrations, available for download at the Technical Resources section of the Stilla Technologies website.

Component Name	Reference	Concentration	Description
Kanamycin Resistance Gene Crystal Digital PCR® Assay	R53000	10X	Detects four zones of Kanamycin resistance gene
KanR Positive Control	R53000.PC0	10X	Contains: Synthetic target sequences (4)

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 61°C for 90 seconds	1°C/sec
Step 4	Temperature 55°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 60 Iterations	-
Step 3.1	Temperature 95°C for 15 seconds	2°C/sec
Step 3.2	Temperature 65°C for 90 seconds	2°C/sec
Step 4	Temperature 55°C for 900 seconds	1°C/sec
Step 5	Release for Ruby Chip	-

Image Acquisition

Download the dedicated scanning file from the Technical Resources section of the Stilla Technologies website:

- ScanningTemplate_Prism6_4C_KanR_R53000_v1.ncx (6-color naica® system)
- NioProtocol_4C-60X-65°C-90s-final-55°C-15min_v1.nioprotocol (Nio™ Digital PCR)
- NioAssay_4C_KanR_R53000_v1.nioassay (Nio™ Digital PCR)

Image Analysis

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

- CompensationMatrix_Prism6_KanR_R53000_v1.ncm (6-color naica® system)
- CompensationMatrix_Nio_KanR_R53000_v1.ncm (Nio™ Digital PCR)
- AnalysisConfiguration_KanR_R53000_v1.nca (all systems)

Consumables Required but Not Provided

- Ruby Chip (C16011)
- naica® PCR MIX 10X (R10106)
- Crystal 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*

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. Examples of results obtained on the Nio™+ system are given below.

Caution: the concentrations estimated by Nio™ Analyzer or by Crystal Miner for the preset populations do not correspond to the concentrations of each DNA fragment, except for the single-positive populations.

To determine the concentration of each DNA fragment, download the dedicated **Excel Analysis Workbook** from the Technical Resources section of the Stilla Technologies website and follow the instructions given on the first worksheet:

- PostProcessing_KanR_R53000.xlsx

Wet lab testing was carried out using DNA representing fragmented and complete DNA of the Kanamycin resistance gene. Representative data is shown in Figure 1.

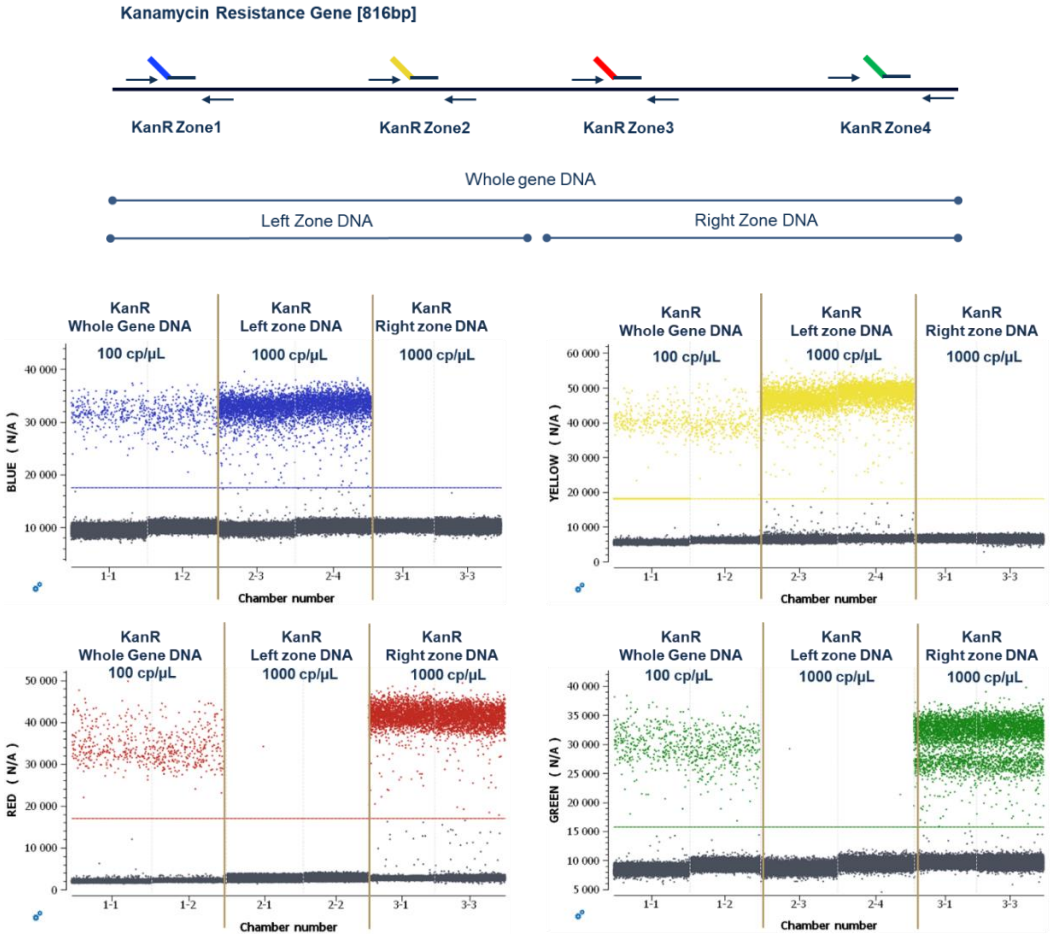


Figure 1: 1D plots obtained during wet lab testing on the Nio™+. *The thresholds are set, using the positive control, at approximately equal distance from the positive and negative clusters.*

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