Crystal Digital PCR® Assay

Information Sheet

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

Product Name

AAV Crystal Digital PCR® Assay (R53002)

Description

Detected AAV Targets

Targets	Sample Type	Detection Channels	Multiplex
AAV Vector Regulatory Elements	DNA	Blue/Green/Red/Infra-Red	Up to 7-plex

The AAV Crystal Digital PCR® Assay is a 10X multiplexed assay designed to detect and quantify key regulatory elements of adeno-associated virus (AAV) vectors, enabling vector integrity assessment using the Ruby Chip. Its flexible mix-and-match feature allows customization across diverse cell and gene therapy workflows.

AAV vectors are essential for efficient gene delivery, stable transgene expression, and safety in gene therapy applications. Their manufacturing and purification require stringent quality control to ensure safe and consistent dosing in both clinical trials and patient treatments, as mandated by regulatory agencies.

This assay delivers a robust and precise solution for vector copy number (VCN) quantification, contamination detection, and viral genome integrity assessment—critical parameters for ensuring batch-to-batch consistency in AAV vector production. An indispensable tool for biopharma manufacturers and researchers, it streamlines QC workflows while supporting regulatory compliance in gene therapy development.

Assay Configuration

The AAV Crystal Digital PCR® Assay configuration is modular, allowing to build an assay according to targets of interest. Select one target per color to combined within a panel for 1-plex up to a 7-plex assay*.

Green	Red	Blue	Infra-Red
ITR2	**WPRE**	**CMV promoter**	**Kanamycin Resistance 3'**
	CMV enhancer		

**More AAV targets available soon.*

Components

AAV Crystal Digital PCR® Assay comprises two types of reagents:

1. A pool of the target specific primers and Crystal Flex Probe. One separate tube is provided per AVV target.
2. Positive Control. Please refer to the lot specific Certificate of Conformity for characterized concentration, available upon request to the Stilla Technologies Technical Support team (support@stilla.fr).

Component Name	Reference	Concentration	Description
AAV cdPCR Assay [Target]	R53002.Target	40X	Contains the pool of specific primers and Crystal Flex Probes for each corresponding AAV target.
AAV Positive Control	R53002.PC0	10X	Contains synthetic target DNA

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 60°C for 30 seconds	2°C/sec
Step 4	Temperature 58°C for 300 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:

- NioProtocol_7C-60X-60°C-30s_v2.nioprotocol (Nio® Digital PCR)
- NioAssay_7C_AAV_v2.nioassay (Nio® Digital PCR)

Image Analysis

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

- CompensationMatrix_Nio_AAV.ncm

Consumables Required but Not Provided

- Ruby Chip (C16011)
- naica® PCR MIX 10X (R10106)
- Depending on the selected AAV targets:
  - Crystal Universal Reporters 3 (R41401 200 reactions, R41402 1000 reactions)
  - 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. Depending on the selected AAV targets, two PCR mix preparation tables are possible. Stilla recommends to prepare a pool for at least 10 chambers.

For AAV targets using **Blue**, **Green**, and/or **Red**:

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 ●	Add from 1 to 3 target assays		
Target blue (optional)	*40x*	*1x*	*0.15*
Target green (optional)	*40x*	*1x*	*0.15*
Target red (optional)	*40x*	*1x*	*0.15*
Crystal Universal Reporter Tube A ●	40x	1x	0.15
Nuclease-free water	NA	NA	Variable
Template DNA, or	**NA**	**NA**	**Variable**
Positive Control Target blue ○	**10x**	**1x**	**0.60**
Positive Control Target green ○	**10x**	**1x**	**0.60**
Positive Control Target red ○	**10x**	**1x**	**0.60**
	Total reaction volume (µL)		***6.0***

Note for Resistance Gene targets: their synthetic DNA Positive Control corresponds to the full sequence (including the 5' and 3' ends) of the resistance gene. Therefore, if both targets (5' and 3') are included in a given assay configuration, the Positive Control should be included only once to obtain the expected quantification.

For AAV targets using **Blue**, **Teal**, **Green**, **Yellow**, **Red**, **Infra-Red**, and/or **Long-Shift**:

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 ●	Add from 1 to 7 target assays		
Target blue (optional)	*40x*	*1x*	*0.15*
Target teal (optional)	*40x*	*1x*	*0.15*
Target green (optional)	*40x*	*1x*	*0.15*
Target yellow (optional)	*40x*	*1x*	*0.15*
Target red (optional)	*40x*	*1x*	*0.15*
Target infra-red (optional)	*40x*	*1x*	*0.15*
Target long-shift (optional)	*40x*	*1x*	*0.15*
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, or	**NA**	**NA**	**Variable**
Positive Control Target blue ○	**10x**	**1x**	**0.60**
Positive Control Target teal ○	**10x**	**1x**	**0.60**
Positive Control Target green ○	**10x**	**1x**	**0.60**
Positive Control Target yellow ○	**10x**	**1x**	**0.60**
Positive Control Target red ○	**10x**	**1x**	**0.60**
Positive Control Target infra-red ○	**10x**	**1x**	**0.60**
Positive Control Target long-shift ○	**10x**	**1x**	**0.60**
		Total reaction volume (µL)	***6.0***

Note for Resistance Gene targets: their synthetic DNA Positive Control corresponds to the full sequence (including the 5' and 3' ends) of the resistance gene. Therefore, if both targets (5' and 3') are included in a given assay configuration, the Positive Control should be included only once to obtain the expected quantification.

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.

Wet lab testing was carried out using synthetic single strand DNA as positive control. Examples of results obtained on the Nio®+ configuration of Nio® Digital PCR are given below.

For integrity analysis, download the dedicated **Excel Analysis Workbook** corresponding to the assay level of plex from the Technical Resources section of the Technologies website and follow the instructions given on the first worksheet:

- PostProcessing_AAV_R53002_2-plex.xlsx
- PostProcessing_AAV_R53002_3-plex.xlsx
- PostProcessing_AAV_R53002_4-plex.xlsx
- PostProcessing_AAV_R53002_5-plex.xlsx
- PostProcessing_AAV_R53002_6-plex.xlsx
- PostProcessing_AAV_R53002_7-plex.xlsx

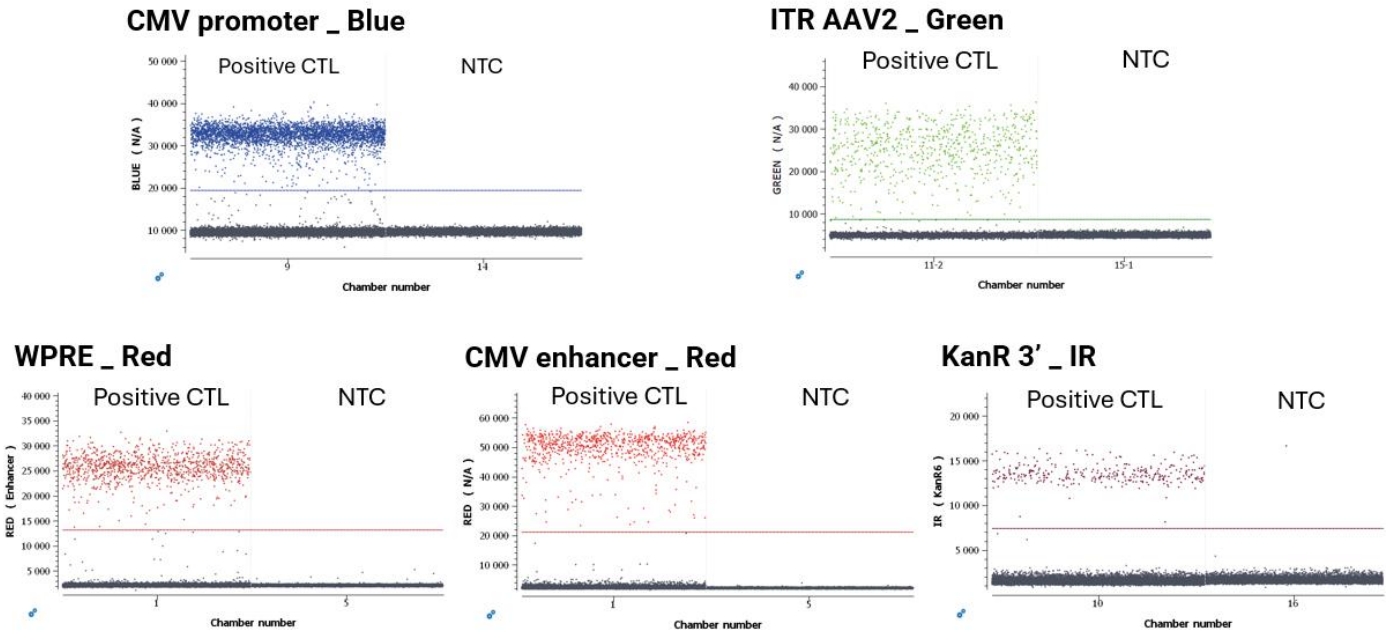

Figure 1: 1D plots obtained with each of the targets of the AAV Crystal Digital PCR® Assay during wet lab testing on Nio® Digital PCR.

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