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/Teal/Green/Yellow/Red/ Infra-Red/Long-Shift	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, which allows 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*.

Blue	Teal	Green	Yellow	Red	Infra-Red	Long-Shift
CMV promoter	**GFP / YFP / CFP**	**ITR2**	**bGH**	**WPRE**	**Kanamycin Resistance 3'**	**Kanamycin Resistance 5'**
hSyn	**-**	**-**	**SV40 polyA**	**CMV enhancer**	**NeoKanamycin Resistance 3'**	**NeoKanamycin Resistance 5'**

**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 Probes. 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 demand to Stilla's Technical Support team at support-stilla@bio-rad.com.

Component Name	Reference	Concentration	Description
AAV cdPCR Assay [Target]	R53002	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	-

Data Acquisition

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

- NioProtocol_7C-60X-60°C-30s+58°C300s.nioprotocol (Nio Digital PCR)
- NioAssay_7C_AAV_R53002.nioassay (Nio Digital PCR)

Data 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 preparing 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*

*Crystal Universal Reporter Tube A must always be included in the reaction mix, even if none of the targets are detected in blue, green, or red for given assay configuration.

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.

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

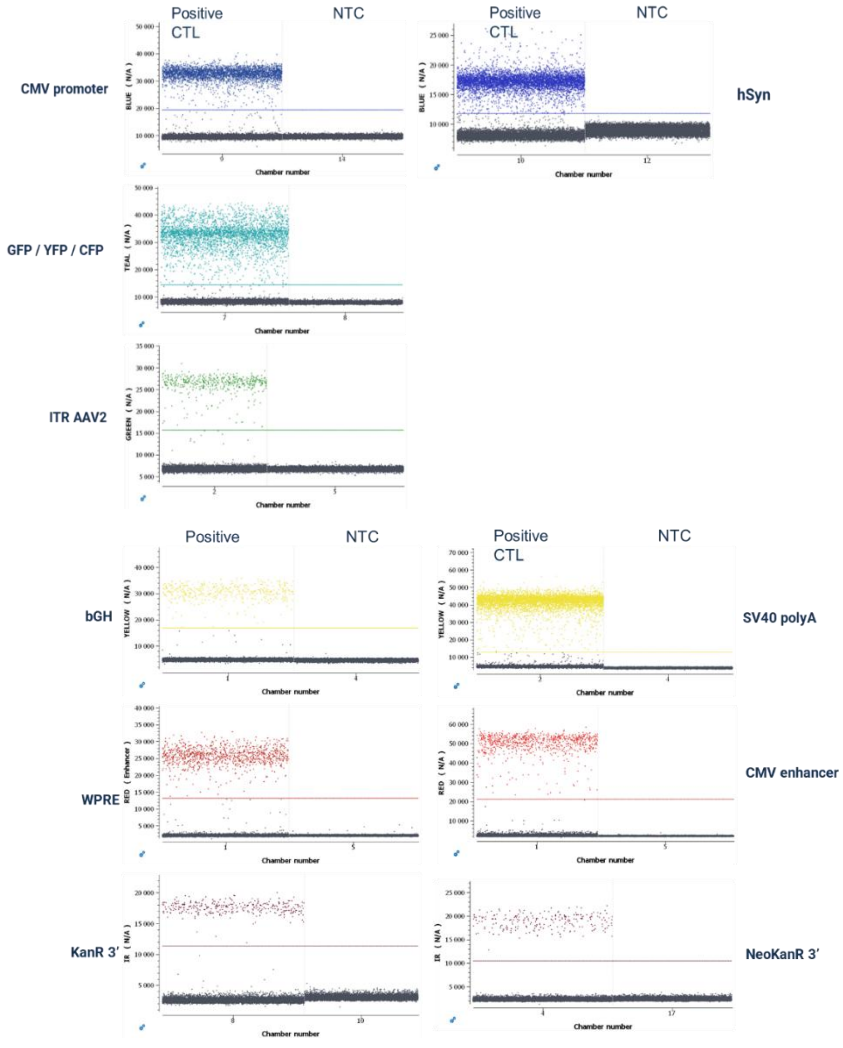
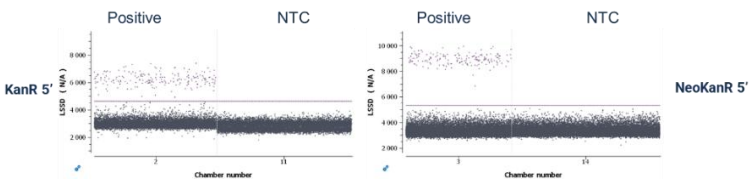


Figure 1: 1D plots obtained with each of the targets of the AAV Crystal Digital PCR® Assay during wet lab testing on Nio Digital PCR.

These data have been obtained using simplex assays. When using multiplex assays (combination of several target assays) separability scores may be different. Stilla recommends validating the assay in multiplex with final samples.



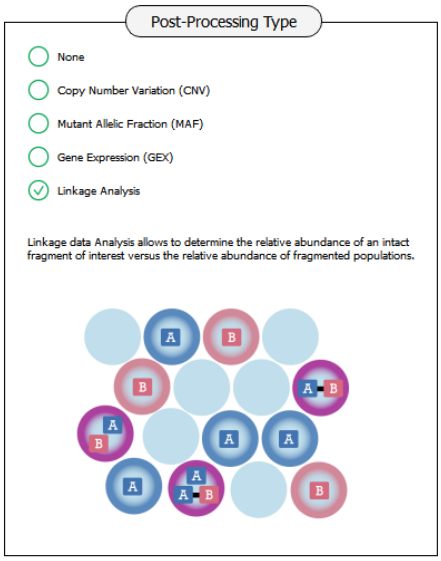
Post-Processing

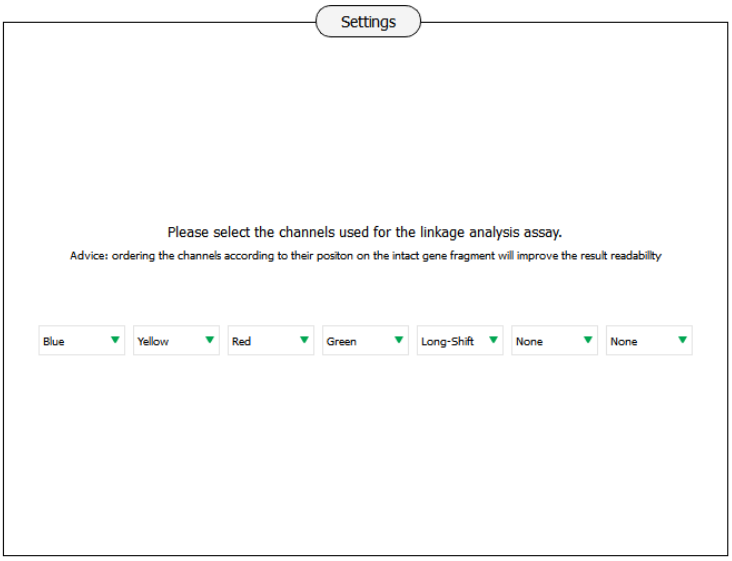
To perform a post-processing analysis of the results, click on “Setup” in the “POST PROCESSING” menu and select the appropriate analysis: Linkage Analysis.

The setting should be adjusted according to the selected AAV targets.

Advice: ordering the channels according to their position on the intact gene fragment will improve the result readability.

An example is given below with 5 AAV targets:





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

AIS_R53002_v4

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