Crystal Digital PCR® Assay

Information Sheet

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

Product Name

CHO Residual DNA Crystal Digital PCR® Assay (R53003)

Description

Detected Target

Target	Sample Type	Detection Channels	Multiplex
CHO GAPDH gene	DNA	Blue/Green/Red	3

The CHO Residual DNA Crystal Digital PCR® Assay is a highly sensitive 10X assay designed to detect and quantify residual DNA from the Chinese Hamster Ovary cells (CHO) host by specifically amplifying a segment of the GAPDH gene. Checking for residual host DNA in bioproduction is crucial to ensure product safety and quality, as leftover host DNA could pose risks such as immunogenicity, oncogenicity, or contamination. That is why regulatory guidelines set strict limits on acceptable levels. Regulatory guidelines typically limit residual host DNA to ≤10 ng per dose and fragment sizes to ≤200 base pairs to minimize safety risks.

Assay configuration

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

Targets	Base changes	Blue	Teal	Green	Yellow	Red	Infra-Red	Long-Shift
CHO GAPDH gene >200bp [>400bp]	N/A	X		X		X		
CHO GAPDH gene >200bp [200bp-400bp]	N/A	X				X		
CHO GAPDH gene <200bp	N/A					X		

Using this assay, three types of GAPDH gene fragments can be detected:

- Short DNA Fragments < 200bp (Red only fragments)
- Medium DNA Fragments from 200 bp to 400 bp (Red and Blue fragments)
- Long DNA Fragments >400 bp (Red, Blue and Green fragments)

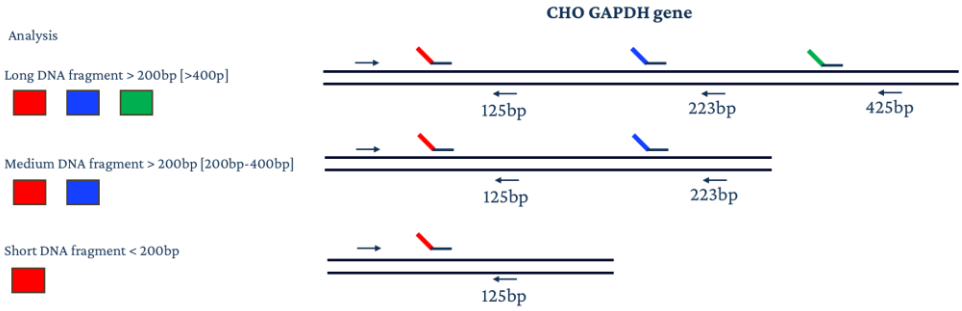

Figure 1: Schematic of the CHO GAPDH gene amplified with the different set of primers. *Each DNA fragment amplified (short, medium and long) will be detected in either one, two or three colors depending on its length.*

Being able to detect and quantify three size fragments can enhance sample analysis and improve the purification process by allowing better assessment of host cell DNA fragmentation.

Components

The CHO Residual DNA Crystal Digital PCR® Assay comprises two reagents: a pool of the assay specific primers and Crystal Flex Probes and a Positive Control. Lot-specific Certificate of Conformity with characterized concentrations will be provided upon request to the Technical Support Team. Please contact us for further assistance

Component Name	Reference	Concentration	Description
CHO Residual DNA Crystal Digital PCR® Assay	R53003	10X	Detects three fragments length of the GAPDH gene (short, medium and long fragments)
CHO Residual DNA Positive Control	R53003.PC0	10X	Contains: synthetic target sequence of the GAPDH gene

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 90 seconds	1°C/sec
Step 4	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 60°C for 60 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:

- ScanningTemplate_Prism3_3C_CHO_Residual_DNA_R53003_v1.ncx (3-color naica® system)
- ScanningTemplate_Prism6_3C_CHO_Residual_DNA_R53003_v1.ncx (6-color naica® system)
- NioProtocol_3C-60X-60°C-60s.nioprotocol (Nio® Digital PCR)
- NioAssay_3C_CHO_Residual_DNA_R53003_v1.nioassay (Nio® Digital PCR)

Image Analysis

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

- CompensationMatrix_Prism3_CHO_Residual_DNA_R53003_v1.ncm (3-color naica® system)
- UniversalCompMatrix_3C_Prism6-Nio.ncm (6-color naica® system, Nio® Digital PCR)
- AnalysisConfiguration_CHO_Residual_DNA_R53003_v1.nca (all systems)

Consumables Required but Not Provided

- Ruby Chip (C16011)
- naica® PCR MIX 10X (R10106)
- Crystal Universal Reporters 3 (R41401 200 reactions, R41402 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
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.

Wet lab testing was carried out using human genomic DNA (hgDNA) or H_2O as negative controls, a positive control consisting of synthetic CHO GAPDH gene DNA (Ctrl+ at 20 cp/µl or 500 cp/µl) and CHO genomic DNA (gDNA).

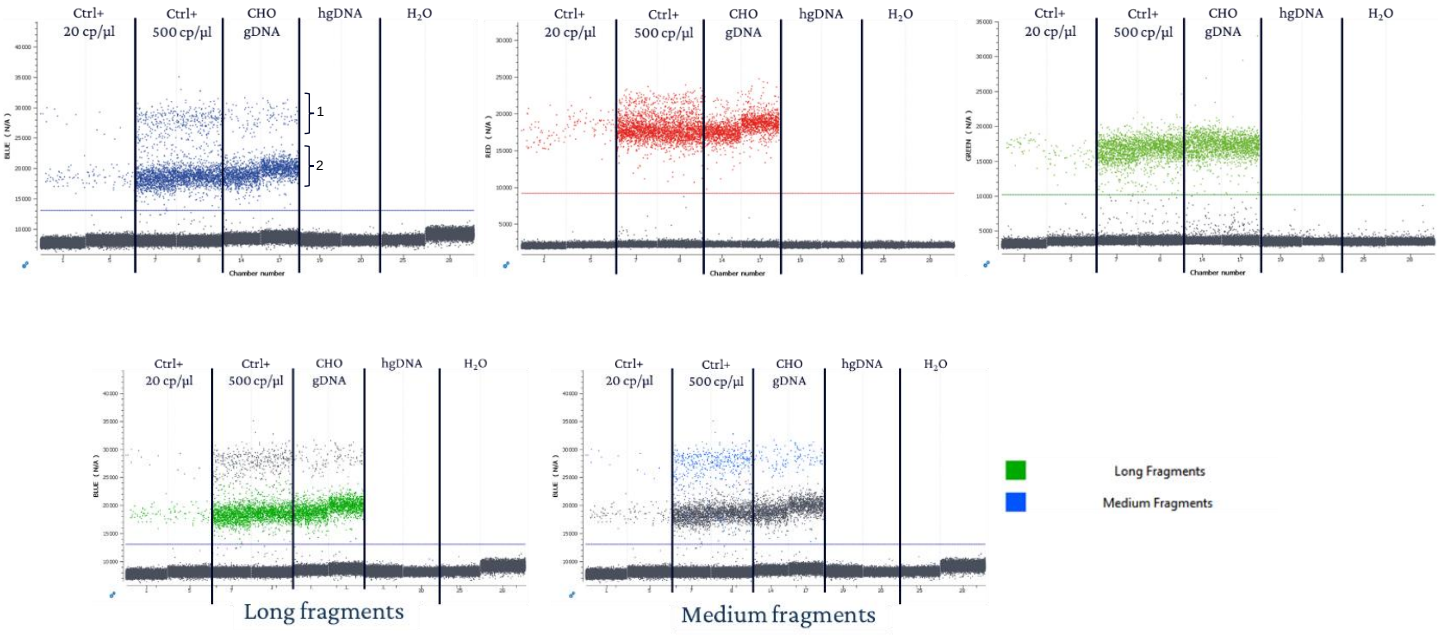

Figure 2: 1D plots obtained during wet lab testing on the Nio®+ system. *The thresholds are set at approximately equal distance from the positive and negative clusters. Note that 2 clusters are detected in blue for the Ctrl+ and the CHO gDNA samples. This is due to a difference in amplification efficiency between the medium and short fragments. Cluster 1 corresponds to the medium fragments, and cluster 2 corresponds to the long fragments.*

To convert copies/µL to ng/dose for 1data analysis: 1 cp/µl = ~2,86 pg/µl for a CHO haploid genome.

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