Crystal Digital PCR® Assay

Information Sheet

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

Product Name

Barley 6plex Crystal Digital PCR® Assay (R54001)

Description

Detected Targets

Targets	Detection Channels	Multiplex
1H2-Barke, 1H2-GP, 1H4-Barke, 1H4-GP, 1H6-Barke, 1H6-GP	Blue / Teal / Green / Yellow / Red / IR	6

The Barley 6plex Crystal Digital PCR® Assay is a highly sensitive 10X assay designed to detect and quantify 6 barley markers used for breed selection.

Assay Configuration

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

Targets	Blue	Teal	Green	Yellow	Red	Infra-Red	Long-Shift
1H2-Barke			X				
1H2-GP		X					
1H4-Barke				X			
1H4-GP						X	
1H6-Barke	X						
1H6-GP					X		

Components

The assay comprises two reagents: a pool of the assay specific primers and Crystal Flex Probes and a pool of 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
Barley 6plex Crystal Digital PCR® Assay	R54001	10X	Detection of the 6 targets 1H2-Barke, 1H2-GP, 1H4-Barke, 1H4-GP, 1H6-Barke, 1H6-GP
Barley 6plex Positive Control	R54001.PC0	10X	Contains synthetic DNA of the 6 targets

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 40 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 55°C for 300 seconds	1°C/sec
Step 5	Release for Ruby Chip	-

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 40 Iterations	-
Step 3.1	Temperature 95°C for 15 seconds	1°C/sec
Step 3.2	Temperature 58°C for 60 seconds	1°C/sec
Step 4	Temperature 55°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-40X-60°C-60s+55°C300s.nioprotocol (Nio Digital PCR)
- NioAssay_6C_Barley_R54001.nioassay (Nio Digital PCR)

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

- ScanningTemplate_Prism6_6C_Barley_R54001_v1.ncx (6-color naica system)

Data Analysis

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

- MeanCompMatrix_Barley_6C_Nio.ncm (Nio Digital PCR)
- AnalysisConfiguration_Barley.nca (Nio Digital PCR)
- MeanCompMatrix_Barley_6C_Naica.ncm (6-color naica system)
- AnalysisConfiguration_Barley_naica.nca (6-color naica system)

Consumables Required but Not Provided

- Ruby Chip (C16011)
- naica® PCR MIX 10X (R10106)
- Crystal Universal Reporters 7 (R42401 200 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).

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 are given below.

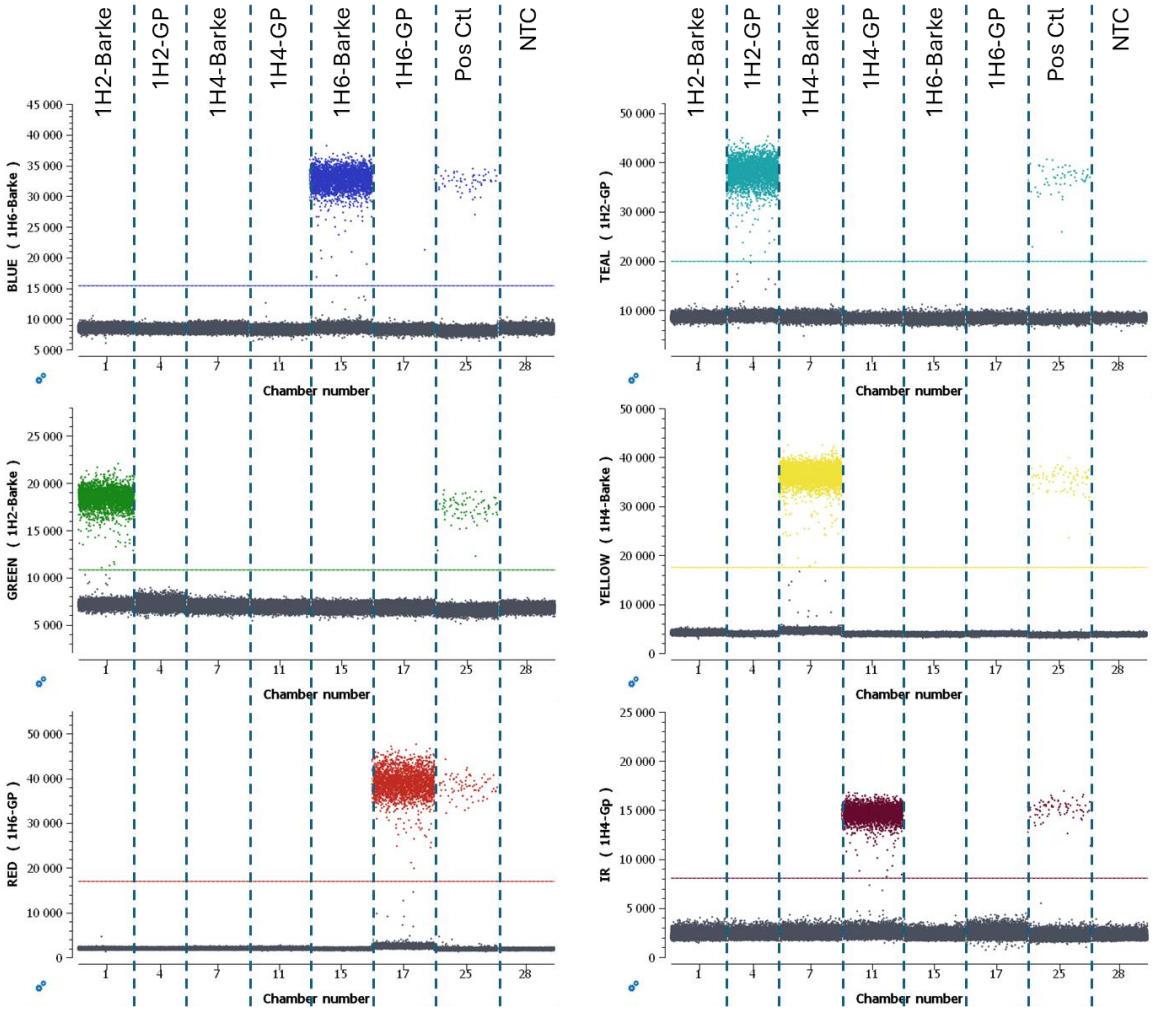

Figure 1: 1D plots obtained during testing on the Nio+. *The thresholds should be set at approximately equal distance from the positive and negative clusters.*

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