Crystal Digital PCR® Assay

Information Sheet

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

Product Name

JAK2 (V617, V617F) Crystal Digital PCR® Assay (R51048)

Description

Detected Target

Targets	Sample Type	Detection Channels	Multiplex
JAK2 (V617, V617F)	DNA	Blue/Green	2

The JAK2 (V617, V617F) Crystal Digital PCR® Assay is a 10X assay designed to detect and quantify 1 mutation in the Janus Kinase 2 (JAK2) gene using the Ruby Chip. JAK2 encodes a non-receptor tyrosine kinase that plays a major role in cytokine and growth factor intracellular signaling. It is involved in several processes such as cell growth, development, differentiation and histone modifications. The JAK2 V617F mutation is associated with hematological malignancies and disorders.

Assay configuration

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

Targets	Base changes	Blue	Teal	Green	Yellow	Red	Infra-Red	Long-Shift
Wild-type (WT) JAK2 V617	N/A	X						
JAK2 V617F	c.1849G>T			X				

Components

The JAK2 (V617, V617F) Crystal Digital PCR® Assay comprises two reagents: a pool of the assay specific primers and Crystal Flex Probes and Positive Control. Please refer to the lot specific Certificate of Conformity for characterized concentration, available for upon request from Stilla’s Technical Support team (support@stilla.fr).

Component Name	Reference	Concentration	Description
JAK2 Crystal Digital PCR® Assay	R51048	10X	Detects 1 mutation in the JAK2 gene
JAK2 Positive Control	R51048	10X	Contains: hgDNA, synthetic JAK2 V617F mutant

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 58°C for 30 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 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:

- ScanningTemplate_Prism3_JAK2_R51048_v1.ncx (3-color naica system)
- ScanningTemplate_Prism6_JAK2_ R51048_v1.ncx (6-color naica system)
- NioProtocol_3C-60X-60°C-30s_v2.nioprotocol (Nio Digital PCR)
- NioAssay_3C_JAK2_ R51048_v1.nioassay (Nio Digital PCR)

Image Analysis

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

- CompensationMatrix_Prism3_JAK2 _R51048_V1.ncm (3-color naica system)
- UniversalCompMatrix_3C_Prism6-Nio.ncm (6-color naica system, Nio Digital PCR)
- AnalysisConfiguration_JAK2_ R51048_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 blue and green 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) and H_2O as negative controls and a positive control (Ctl +) consisting of hgDNA and synthetic JAK2 V617F mutant.

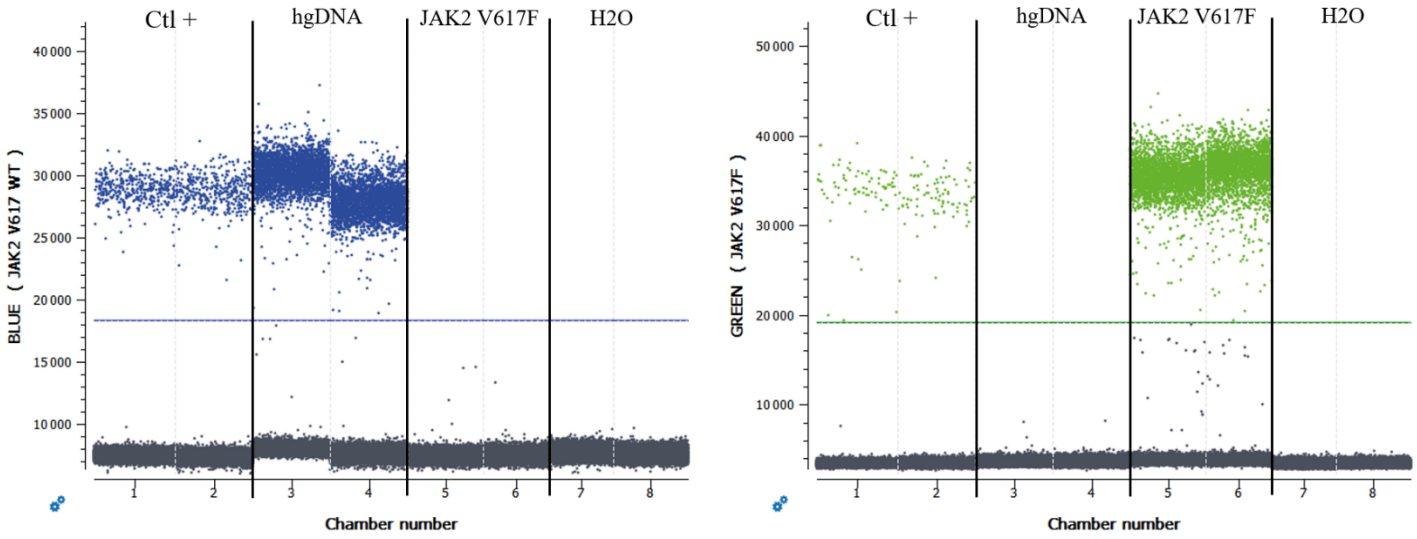

Figure 1: 1D plots obtained during wet lab testing on the Nio+. *The blue and green thresholds are set at approximately equal distance from the positive and negative clusters. Note: a slight non-specific interaction of the blue Crystal Flex Probe on the JAK2V617F template in the blue channel, and of the green Crystal Flex probe on JAK2V617 WT template in the green channel can be observed.*

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