

Crystal ddPCR Assays

Information Sheet

June 2026

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

Product Ordering Information

JAK2 (V617, V617F) ddPCR Assay (R51048)

Catalog No.	Product Ref No.	Product Description
12026921	R51048.1	JAK2 V617 V617F ddPCR Assay 200R
12026922	R51048.2	JAK2 V617 V617F ddPCR Assay 1000R

Purpose

This document provides additional information for using JAK2 V617 V617F ddPCR Assay. Refer to the Crystal Digital PCR Assay for use with Crystal Universal Reporters Instructions for Use for general guidance.

Intended Product Use

JAK2 V617 V617F ddPCR Assay is intended for the in vitro detection and quantification of the JAK2 V617F mutation (c.1849G>T, p.Val617Phe) in human DNA samples, including genomic DNA and highly fragmented DNA matrices such as FFPE-derived DNA or circulating cell-free DNA (cfDNA).

This assay is designed for use by laboratory professionals in a research setting. It is intended to be used exclusively with Crystal Universal Reporters on Nio and Naica Digital PCR Systems, in conjunction with the Crystal Digital PCR Assay Instructions for Use. It is not intended to be used with QX700 ddPCR Systems.

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Description

Detected Target

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

The JAK2 V617 V617F ddPCR Assay is a two-plex, 10X concentrated assay based on droplet digital PCR (ddPCR) technology. It enables simultaneous detection and absolute quantification of wild-type Janus Kinase 2 (JAK2) gene and 1 mutation V617F (c.1849G>T) in a single reaction, using the Blue and Green fluorescence channels respectively.

The assay utilizes sequence-specific primers and Crystal Flex Probes in combination with Crystal Universal Reporters, and is optimized for use with RDG16 cartridges on the Nio and Naica Digital PCR platforms. It supports mutant allelic fraction (MAF) calculation via the NioAnalyzer post-processing module.

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 ddPCR 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 upon demand to our Technical Support team

Component Name	Reference	Concentration	Description
JAK2 ddPCR Assay	R51048	10X	Detects 1 mutation in the JAK2 gene
JAK2 Positive Control	R51048.PC 0	10X	Contains: hgDNA, synthetic JAK2 V617F mutant

Thermocycling Programs

On the naica system

Step		Ramp rate
Step 1	Partition for RDG16 Cartridges	-
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 RDG16 Cartridges	-

On the Nio Digital PCR

Step		Ramp rate
Step 1	Partition for RDG16 Cartridges	-

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 RDG16 Cartridges	-

Data Acquisition

Download Nio dedicated technical files from [bio-rad.com](https://www.bio-rad.com).

- NioProtocol_3C-60X-60°C-30s+58°C300s.nioprotocol (Nio Digital PCR)
- NioAssay_3C_JAK2_R51048.nioassay (Nio Digital PCR)

Download naica dedicated technical files from [bio-rad.com](https://www.bio-rad.com).

- ScanningTemplate_Prism3_JAK2_R51048_v1.ncx (3-color naica system)
- ScanningTemplate_Prism6_JAK2_R51048_v1.ncx (6-color naica system)

Data 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

- RDG16 Cartridges, pack of 12 (12025252)
- naica 10X ddPCR Mix, 2 × 0.375 mL (12025258)
- Crystal Universal Reporters 3 (12026172 (200 rxn), 12026157 (1000 rxn))
- 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 ddPCR Mix Buffer A	10x	1x	0.60
naica ddPCR Mix Buffer B	100%	4%	0.24
Crystal ddPCR 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
<i>or Positive Control</i>	10x	1x	0.60
<i>Total reaction volume (µL)</i>			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 using 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 ddPCR 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/ μ L. 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 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 H₂O as negative controls and a positive control (Ctl +) consisting of hgDNA and synthetic JAK2 V617F mutant.

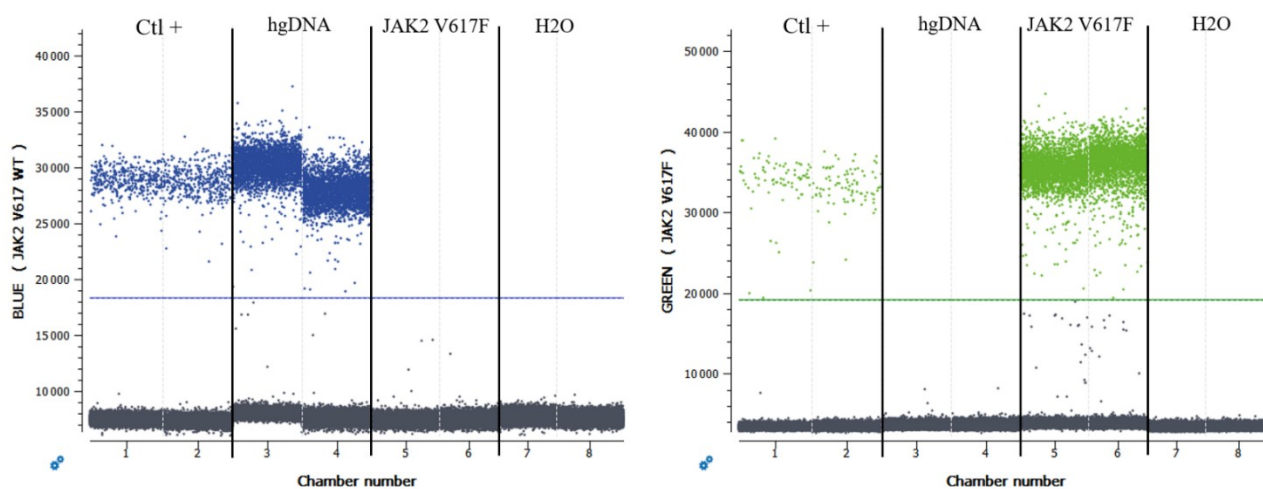


Figure 1: 1D plots obtained during wet lab testing on the Nio+. The blue and green thresholds are set at approximately equal distance from the positive and negative clusters. Note: a slight non-specific interaction of the blue Crystal Flex Probe on the JAK2V617F template in the blue channel, and of the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

Post-Processing (only available with NioAnalyzer software)

To perform a post-processing analysis of the results, click on “Setup” in the “POST PROCESSING” menu and select the appropriate analysis: **Mutant Allelic Fraction (MAF)**.

Follow

specific

instru

Add population to processing

All populations should be added to processing, and “B_JAK2WT” selected as reference.
Clicking on apply will launch the calculation. The values will be displayed in the “Results” tab.

Revision History

Release Date	SAP DIR and Version	Description
June 2026	10000260763 Ver A	Reformat/edit document using Bio-Rad branding

Bio-Rad Technical Support

The Bio-Rad Technical Support department in the U.S. is open Monday through Friday, 5:00 AM to 5:00 PM, Pacific time.

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For technical assistance outside the U.S. and Canada, contact your local technical support office or click the Contact Us link at bio-rad.com.

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