OncoPlex® *JAK2* Mutation Kit

Catalogue # MMD004

FOR RESEARCH USE ONLY

Multiplex Mutation Detection (MMD) real-time PCR assay for the detection of V617F (GTC>TTC) mutation in human *JAK2* gene

Store at -20°C

Protect from light

Instructions for Use – English

Version 7.0 – June 2025

genefirst

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1. Glossary

Abbreviation	Definition
PCR	Polymerase Chain Reaction
MMD	Multiplex Mutation Detection
PC	Positive Control
NC	Negative Control
UNG	Uracil-N-Glycosylase
LoD	Limit of Detection
NTC	No Template Control
RUO	Research Use Only
Ct	Cycle Threshold
JAK2	Janus Kinase 2

2. Copyright and Trademarks

3. Kit Contents

3.1. Materials supplied with the kit:

The kit contains the following four (4) components which are required to complete this protocol.

Tube cap colour	Reagent	Description
Blue	Master Mix	GeneFirst enzyme and buffer mix
Amber	Working Mix	Primers and probes
Yellow	Negative control	Wild type human control DNA (ROX)
Red	Positive control	V617F positive control DNA (FAM)

3.2. Additional Equipment and Reagents Required (not provided in the kit):

- Reagents and equipment for specimen collection, filtration, and DNA extraction
- Distilled water (molecular biology grade)
- DNase, RNase and human DNA-free pipette tips with aerosol barriers
- DNase, RNase and human DNA-free tubes for preparing Reaction Mix
- Pipettes (adjustable)
- Tube racks
- Vortex mixer
- Microcentrifuge
- Cool racks or ice
- Real-Time PCR System
- PCR tubes, plates and accessories compatible with the use of the chosen Real-Time PCR System

4. Shipment and Storage

Oncoplex® *JAK2* Mutation Kit is shipped frozen using gel packs.

Upon receipt of the kit, the components must be stored in a freezer at -20°C or below.

The contents must be protected from light to prevent photobleaching and must be stored in the manufacturer's packaging.

Provided that the components have been stored correctly according to the recommendations, the Oncoplex® *JAK2* Mutation Kit is stable until the expiration date indicated in the packaging.

5. Introduction

The GeneFirst Oncoplex® *JAK2* Mutation Kit is a real-time PCR-based test designed for the detection of a mutation in the human *JAK2* gene in DNA extracted from blood sample material.

The Oncoplex® *JAK2* Mutation Kit enables, in one closed-tube reaction, the detection of the V617F (GTC>TTC) activating mutation against a background of wild-type genomic DNA using a real-time PCR assay based on GeneFirst's proprietary MMD-PCR technology. The kit detection limit is 1% of the mutant allele in a background of wild-type genomic DNA in assays utilising between **30–500 ng** of input DNA.

5.1. Intended Use

The Oncoplex® *JAK2* Mutation Kit is an *in vitro* real-time PCR test for the detection of the V617F (GTC>TTC) activating mutation in the human *JAK2* gene.

5.2. Target Environment

The Oncoplex® *JAK2* Mutation Kit may be used to detect *JAK2* mutations in pathology laboratories or epidemiological studies in research settings. The assay is sold as an RUO reagent.

5.3. Principle

The Oncoplex® *JAK2* Mutation Kit relies on a combination of specially designed mutation-specific primers, blockers, and probes, and unique cycling conditions to facilitate selective amplification of the PCR product from the mutant allele, while simultaneously blocking amplification from the wild-type (normal) allele. This allows the detection of rare mutations against a high background of normal, non-mutated genomic DNA (Figure 1).

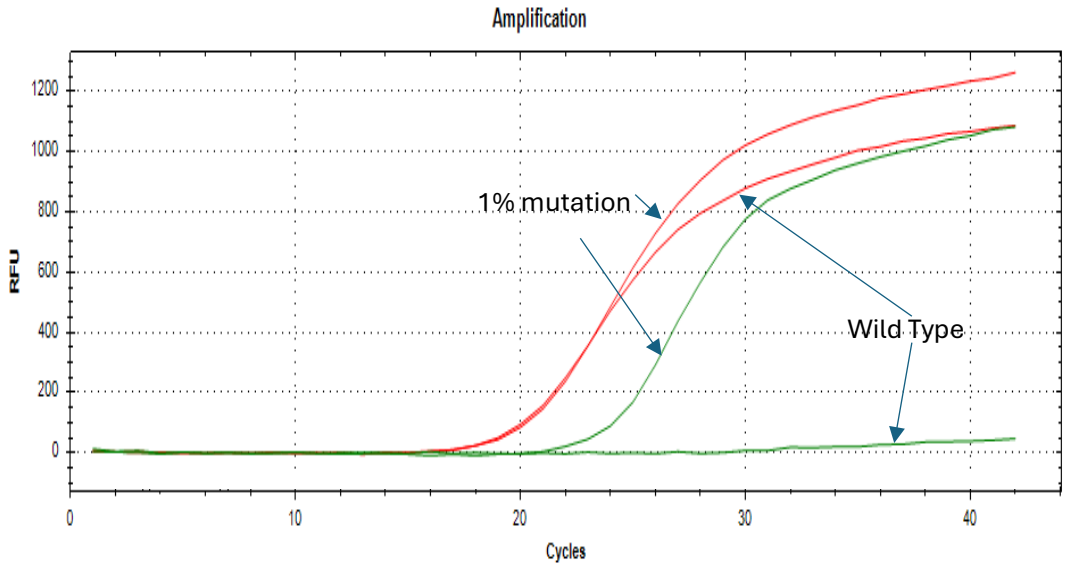


Figure 1: Representative amplification curves in FAM channel for JAK2 1% mutant and wild type DNA (green). Representative amplification curves in ROX channel for Internal Control, (1% mutant and wild type) (red). Y axis = Delta Rn, X-axis = cycle number

5.4. Controls Provided in the Kit

The tube with a **red** cap contains a Positive Control (PC) to be used in each PCR run. The PC contains mutated *JAK2* DNA in the background of the wild-type human DNA.

The tube with a **yellow** cap contains a Negative Control (NC) to be used in each PCR run. The NC contains wild-type human DNA.

The assay contains an internal control labelled with the ROX fluorophore, which amplifies a fragment of the human *KRAS* gene. The amplification of this control confirms the absence of PCR inhibitors in the sample and that the input DNA is of sufficient quality.

6. Contraindications, Warnings, and Precautions

6.1. Contraindications

There are no known contraindications identified.

6.2. Interfering Substances

The performance of the kit may be adversely affected by known PCR inhibitors co-extracted from patient samples (such as blood, paraffin, or formalin).

6.3. Warnings and Precautions

- This kit is designed to be used for *in vitro* detection in a research setting and should be used by trained personnel with good laboratory practice and good competency in real-time PCR
- Upon arrival, please check the kit for any signs of damage. If damaged, please contact the GeneFirst customer service or the local distributor. Do not use damaged kit components as they may not give the expected performance
- The kit is not intended to be re-used
- Do not use the product beyond its expiry date
- Do not mix reagents from different batches
- Negative and positive controls provided in the kit must be used in each experiment and allow for easy troubleshooting
- Fluorescently labelled probes included in the Amber tube (Working Mix) are sensitive to photobleaching. Exposure to light should be avoided as much as possible
- Lab coats and powder-free gloves must be worn at all times
- Never touch the inside of the tube cap
- Appropriate pipette tips with aerosol barrier must be used that are free of DNase, RNase and human DNA
- Use appropriate measures to decontaminate working surfaces such as wipe/spray with 0.5% Sodium Hypochlorite solution or DNA AWAY™
- Thaw all components thoroughly at room temperature before using the kit, mix and keep on ice
- Avoid excessive vortexing
- Disposal of unused reagents and waste must be done in accordance with country or local regulations
- Safety Data Sheet (SDS) is available on request from either GeneFirst or your distributor

7. Operating Procedure

7.1. PCR Reaction Mix Setup

Prepare the PCR Reaction Mix according to the table below for the required number of reactions (including an excess). Perform all steps on ice or at room temperature, with minimal exposure to light. Before use, the Master Mix and Working Mix should be fully thawed and mixed thoroughly by vortexing and brief centrifugation.

Optionally, more sample DNA and control may be added (up to 6 µl) if needed. The water volume should then be adjusted accordingly to obtain a total reaction volume of 20 µl.

Table 1: Summary of Components Required for PCR

Tube cap colour	Name	Volume per single reaction
Blue	Master Mix	**8 µl**
Amber	Working Mix	**6 µl**
(Not supplied with the kit)	H_2O (molecular biology grade)	**To 19 µl (0-5 µl as required)**
	And	
Red	Positive Control	**1 µl**
	Or	
Yellow	Negative Control	**1 µl**
	Or	
NTC	No Template Control	**1 µl**
	Or	
N/A	Test sample	**1-6 µl**
	Total	**20 µl**

NOTE: The Master Mix contains Uracil-N-Glycosylase (UNG) and dUTP to prevent any carry-over contamination.

Transfer 19 µl of the Reaction Mix into each of the wells of a PCR plate, and then add 1 µl of either PC, test sample, or NC into each well. It is recommended that at least one PC, one NC, and one NTC be included in each run.

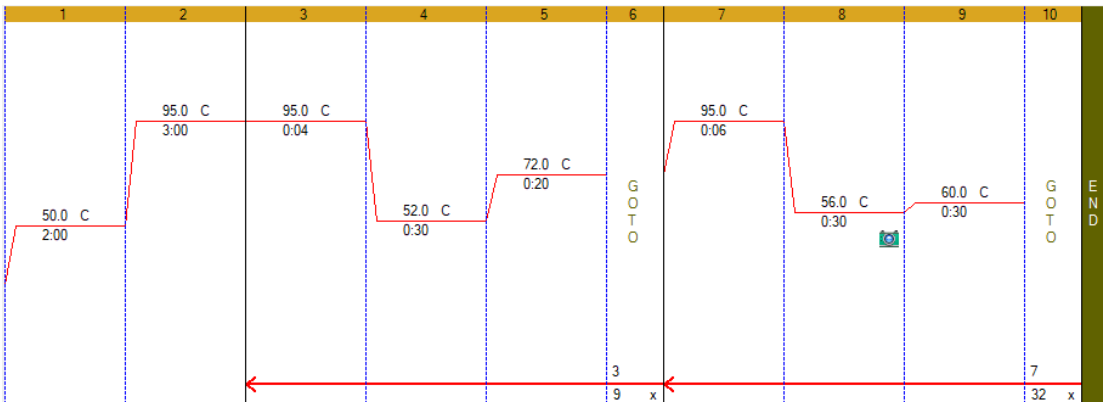
Seal the PCR plate and spin it down briefly. Each well should be sealed tightly to avoid evaporation.

7.2. Real-Time PCR Instrument Settings

The PCR cycling conditions shown in Table 2 should be used:

Table 2: PCR Cycling Conditions for *JAK2* Assay

Stage	Cycles	Temperature (°C)	Duration	Data collection
1- Activation stage	1	50	2 min	
		95	3 min	
2 - Amplification stage	10	95	4 secs	
		52	30 secs	
		72	20 secs	
3 – Amplification stage	33	95	6 secs	
		56	30 secs	End-point point FAM and ROX fluorescence collection
		60	30 secs	



The above run settings are for the Bio-Rad CFX96 and Bio-Rad CFX Opus instruments. If other instruments are used, the kit must be validated by the customer prior to use.

Bio-Rad CFX Manager v3.1

- Set the PCR volume to 20µl
- Select "All Channels" to be scanned

8. Data Analysis

8.1. Setting Baseline, Threshold and Ct Calling

Obtain Ct (threshold cycle) values for mutations (FAM) and internal control reactions (ROX) by using the automatic baseline setting or after manual adjustment to 3-15 cycles (start and end respectively) or any other cycle range which excludes background but does not overlap amplification.

The thresholds should be set above the background amplification in the Negative Control (NC). Below are the recommended ranges for the **Bio-Rad CFX Manager v3.1**.

- 100 - 200 RFU for FAM
- 50 – 200 RFU for ROX

8.2. Determine Mutations Presence in Test Sample

Data analysis is performed by assessing the presence of amplification curves for FAM and ROX signals, and the corresponding Ct values. The FAM signals indicate the mutation status of the sample and the ROX signals indicate the internal control status. The run controls (positive control, negative control, and NTC) and the internal control within each reaction mix are assessed to ensure that acceptable Ct values are met and reactions are performing correctly.

To assess the mutation status of each sample, the ΔCt value is calculated using the following formula:

Mutation (FAM) Ct - Internal Control (ROX) Ct = ΔCt

Samples are classed as "mutation-positive" when ΔCt is lower than the cut-off values of **7 cycles**.

8.2.1. Validity of Test and Interpretation of Results

The test is considered valid only if:

- NTC does not show amplification in any channels (FAM and ROX), Ct value is undetermined or is ≥ 30.0
- Negative control shows amplification in ROX channel
- Positive control shows amplification in ROX channel, ΔCt is below 7 Ct
- Amplification of Internal Control in ROX must have Ct values ≤29.0

See the below table for example interpretations

Table 3: All Possible Reaction Outcomes and Result Interpretation

ROX	FAM	Validity	ΔCt	*JAK 2* Mutation
≤29	≤32	Valid	<7	Positive
			≥7	Negative
	>32 or no Ct	Valid	any	Negative
>29 or No Ct	any	Invalid	any	Too little DNA, repeat with more DNA

NOTE: Above these specified ΔCt values, the sample would be reported as "mutation-negative (no mutation detected)", as it may either contain less than the percentage of mutations that can be detected by the kit (beyond the limit of the assay) or the sample is truly mutation-negative.

9. Troubleshooting

Should you encounter problems, please consult the following table.

Observation	Probable Cause	Solution
Absence of amplification	Presence of PCR Inhibitors	The performance of the kit may be adversely affected by known PCR inhibitors co-extracted from patient samples (such as blood, paraffin or formalin) We suggest repeating the test or obtaining a new patient sample/DNA extraction.
	Not enough human DNA in the sample	Repeat the same sample with a higher test sample volume or obtain a new patient sample.
	No human DNA has been extracted	Repeat processing the same sample or obtain a new patient sample.
	Instrument faulty	Check the instrument calibration records and confirm it is working.
	Kit stored at the wrong temperature or under the wrong conditions	Please check the storage temperature and if the contents were exposed to direct sunlight for a long time. Also, ensure to keep the reagents on ice during use and avoid excessive vortexing.
	Incorrect PCR cycling parameters	Verify that PCR cycling parameters correspond to those recommended in the IFU.

10. Specifications of OncoPlex® *JAK2* Mutation Kit

Specification	Description
Technology	MMD-PCR
Target Sequence	Fragment of human *JAK2* gene covering parts of exon 14 and intron 14-15
Limit of Detection	1% mutant allele in samples with 30-500 ng total DNA
Extraction/Inhibition Controls Included	Internal human DNA control for confirming the absence of PCR inhibitors, sample adequacy and quality of DNA extraction
Kit Storage	-20°C
Real-Time PCR Instruments	Bio-Rad Laboratories C1000/CFX96 Touch Real-Time PCR System or CFX Opus
Quality Control	Positive and negative control of amplification
Certification	RUO

11. Symbols

Symbol	Description	Symbol	Description
	Consult instructions for use		Upper limit of storage temperature
	Catalogue number		Keep away from light
	Date of manufacture		Batch code
	Manufacturer		Number of supplied reactions
	Use-by-date		Do not use if package damaged

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12. Customer Contact Information

For all sales order processing, training, and technical support enquiries, please contact the following.

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