JAK2 V617F assay for Countable PCR

DESCRIPTION

Why this assay matters

The *JAK2* V617F mutation is a well-characterized biomarker commonly studied in blood cancer research, such as myeloproliferative neoplasms. Sensitive, reproducible detection enables high-confidence biomarker assessment with every run.

The Countable *JAK2* V617F assay delivers:

- Rapid setup — pre-verified, ready to use, and compatible with Universal Multiplex (UM) chemistry.
- Consistent performance — sensitive enough to detect rare molecules, precise enough for reproducible results every time.

This assay is part of Countable Labs' Community Assays, a collection of ready-to-run assays shared with the scientific community to accelerate discovery. For the *JAK2* V617F assay, please contact us at hello@countablelabs.com for information on the full primer sequences. All performance data was generated using the Countable platform.

Targets

The *JAK2* V617F assay is designed to detect and quantify the *JAK2* V617F mutation compared to the wild-type (WT) human *JAK2* gene with the following specifications on the Countable platform.

Targets	Amplicon length	Probe
JAK2 V617F	88 bp	FAM
JAK2 WT		HEX

Control sample

The following synthetic dsDNA template, spiked into human genomic DNA from cell line GM12878 was used to establish the *JAK2* V617F assay performance and is also recommended to use as a Training Sample during your Countable PCR setup. While not required for running the assay, using this control helps:

- Improve the specificity of your counts
- Serve as a quick check that your setup is performing optimally
- Provide a reference point for monitoring consistency across runs

```
> Template_sequence_JAK2_V617F
AAGCTTTCTCACAAGCATTTGGTTTTAAATTATGGAGTATGTTTCTGTGGAGACGAGAGTAAGTAAAACTACAGGCTTTC
TAATGCCT
```

PERFORMANCE DATA

Assay signal distribution

Clear separation between the target signal and the background is critical for accurate calls. The *JAK2* V617F assay consistently achieves an Intensity Distribution (ID) score above 90 — signifying that the data has a clean distinction between “signal” and “noise,” reducing false positives and improving confidence in quantification.

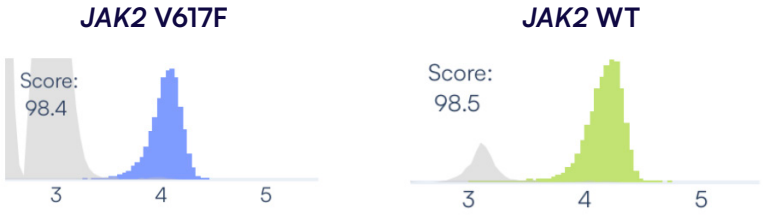

Figure 1. Representative fluorescence intensity histogram for the Countable *JAK2* V617F assay, showing clear separation between target signal (*JAK2* V617F: blue; *JAK2* WT: green) and background (grey). The Intensity Distribution (ID) score for each target appears in the upper-left corner, demonstrating strong signal-to-noise performance.

Instant multiplexing

The *JAK2* V617F assay demonstrates consistent multiplex performance, yielding equivalent Counts per 50 µL whether targets are run individually (single probe mix) or together (double probe mix). This robust performance saves you time and ensures reliable results when running multiple targets.

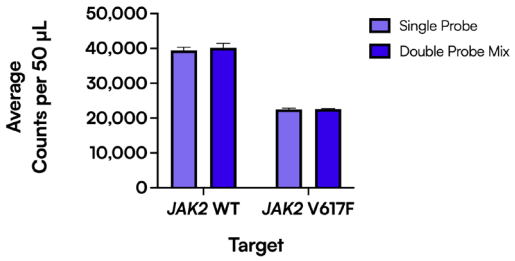

Figure 2. Consistent multiplex assay performance of the Countable *JAK2* V617F assay. Bars represent individual Counts per 50 µL for each reaction, amplified using the synthetic training sample with either a single (1-plex; n=3) or two primer pairs (2-plex; n=3).

Linearity and sensitivity

A dilution series of the control sample shows excellent linearity across a broad dynamic range, demonstrating quantification abilities from high to very low target concentrations.

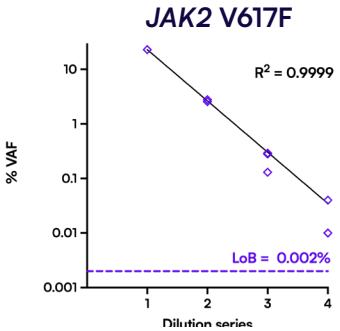

Figure 3. Linearity and limit of blank (LoB) for the Countable *JAK2* V617F assay. A 3-fold dilution series of the synthetic training sample (n=4) shows excellent linearity (R^2 = 0.9999) across the dynamic range. The dashed line marks the LoB for each target, highlighting the assay's ability to detect even rare targets with accuracy.

Precision

Across the assay's full dynamic range, the %CV remains low, delivering consistent and reproducible results. This precision enables confident detection of *JAK2* V617F across a wide range of inputs, ensuring sensitive and reliable biomarker measurement every time.

Dilution	**1**	**2**	**3**
JAK2 V617F counts	6,767	630	55
JAK2 WT counts	22,702	23,011	22,179
Average % VAF	22.96%	2.66%	0.25%
% CV	0.14%	6.18%	30.78%

Table 2. *JAK2* V617F assay precision across the dynamic range.

Summary

The *JAK2* V617F assay delivers sensitive, reproducible results across a wide dynamic range — giving you high-quality biomarker data from the very first run.

Countable Labs.

JAK2 V617F assay

Getting started

This protocol describes the setup and execution of the Countable *JAK2* V617F assay for detecting the *JAK2* V617F mutation and the wild-type (WT) human *JAK2* gene on the Countable PCR platform using hydrolysis probe chemistry.

Detected targets

Targets	Amplicon length	Probe
JAK2 V617F	88 bp	FAM
JAK2 WT		HEX

Materials

Listed below are the materials needed for setting up the amplification mix of this specific assay. Refer to Countable PCR™ Reaction Preparation User Guide (IFU004 Rev 1.0) for the complete list of required materials to set up a Countable PCR reaction.

- ☐ **4X Countable PCR Mix** (Required)
  Cat #: KT0004 (PR0004)
- ☐ ***JAK2* V617F 50x oligo mix** (Required)
  Visit website for sequences
- ☐ **Control sample** (Optional*)
  Visit website for sequences

* The use of a training sample in the Countable system enhances the specificity of counts, verifies assay performance, and can serve as a control, particularly for detecting rare molecules.

Countable PCR reaction set-up

The table below lists the setup of the amplfiication mix specific to this assay. Refer to Countable PCR™ Reaction Preparation User Guide (IFU004) for complete setup instructions.

Reagents	Cat #	Per 50 µL reaction	Final conc.
Nuclease-free water	—	To 50 µL	—
4X Countable PCR Mix	KT0004 (PR0004)	12.5 µL	1X
50X oligo mix*	—	1 µL	1X
Template	—	Variable	—

* Refer to IFU004, Appendix E for details

Thermal cycling conditions

Ensure ramp rate setting of 2 °C/sec. Set the sample volume to 125 µL and the heated lid to 105 °C.

Cycle	Step	Temp (°C)	Time (mm:ss)
1	Initial denaturation	95 °C	02:00
30	Denaturation	95 °C	00:20
	Annealing & extension	60 °C	01:00
1	Hold	4 °C	∞

Visit countablelabs.com for the assay sequences.