

KIT D816V assay for Countable PCR

DESCRIPTION

Why this assay matters

The *KIT* D816V mutation is a key biomarker present in the majority of systemic mastocytosis cases. Sensitive, reproducible detection enables high-confidence biomarker assessment with every run.

The Countable *KIT* D816V 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 *KIT* D816V 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 *KIT* D816V assay is designed to detect the *KIT* D816V mutation and wild-type (WT) human *KIT* gene with the following specifications on the Countable platform.

Targets	Amplicon length	Probe
<i>KIT</i> D816V	89 bp	FAM
<i>KIT</i> WT		HEX

Control sample

The following synthetic dsDNA template, spiked into genomic DNA from cell line GM12878 was used to establish the *KIT* D816V 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_KIT_D816V
CTCATGGTCGGATCACAAAGATGATTTGGTCTAGCCAGAGTCATCAAGAATGATTCTAATTAGTTAAAGGAAACGTGAGTACC
CATTTC
```

PERFORMANCE DATA

Assay signal distribution

Clear separation between the target signal and the background is critical for accurate calls. The *KIT* D816V 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.

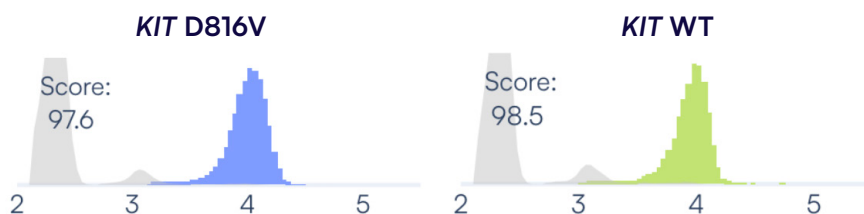


Figure 1. Representative fluorescence intensity histogram for the Countable *KIT* D816V assay, showing clear separation between target signal (*KIT* D816V: blue; *KIT* WT: green) and background (grey). The Intensity Distribution (ID) score for each target appears in the upper-left corner, demonstrating strong signal-to-noise performance.

Instant multiplexing

The *KIT* D816V assay demonstrates consistent multiplex performance, yielding equivalent Counts per 50 μ L whether targets are run individually (1-plex) or together (2-plex). This robust performance saves you time and ensures reliable results when running multiple targets.

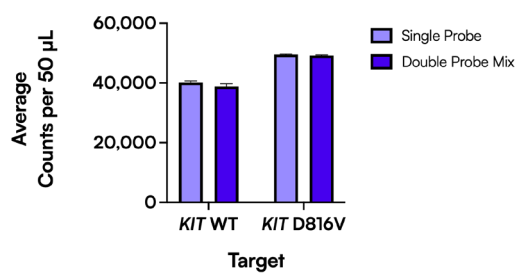


Figure 2. Consistent multiplex assay performance of the Countable *KIT* D816V assay. Bars represent individual Counts per 50 μ L for each reaction, amplified using the synthetic training sample with either a single (1-plex; n=3) or two primer pairs (2-plex; n=3).

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.

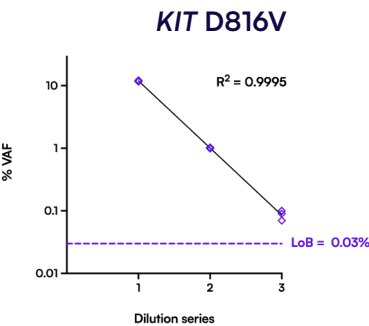


Figure 3. Linearity and limit of blank (LoB) for the Countable *KIT* D816V assay. A 3-fold dilution series of the synthetic training sample (n=4) shows excellent linearity ($R^2 = 0.9995$) across the dynamic range. The dashed line marks the LoB for each target, highlighting the assay's ability to detect 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 *KIT* D816V across a wide range of inputs, ensuring sensitive and reliable biomarker measurement every time.

Dilution	1	2	3
<i>KIT</i> D816V counts	13,594	960	87
<i>KIT</i> WT counts	100,258	94,129	97,925
Average % VAF	11.94%	1.01%	0.09%
% CV	2.09%	1.76%	15.52%

Table 2. *KIT* D816V assay precision across the dynamic range.

Summary

The *KIT* D816V assay delivers sensitive, reproducible results across a wide dynamic range — giving you high-quality biomarker data from the very first run.

KIT D816V assay

Getting started

This protocol describes the setup and execution of the Countable *KIT* D816V assay for detecting the *KIT* D816V mutation and the wild-type (WT) human *KIT* gene on the Countable PCR platform using hydrolysis probe chemistry.

Detected targets

Targets	Amplicon length	Probe
<i>KIT</i> D816V	89 bp	FAM
<i>KIT</i> (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)
- ☐ ***KIT* D816V 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 amplification 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 each	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
40	Denaturation	95 °C	00:20
	Annealing & extension	60 °C	01:00
1	Hold	8 °C	∞