Countable Labs.

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
KIT D816V	89 bp	FAM
KIT (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
30	Denaturation	95 °C	00:20
	Annealing & extension	63 °C	01:00
1	Hold	8 °C	∞

Visit countablelabs.com for the assay sequences.