Plentiplex™ Mastocytosis

Made by PentaBase

DISPENSE READY QUICK GUIDE

Plentiplex™ Assay for Sensitive Detection of the *KIT* D816V Mutation

#Cat. No.: 7023-7024

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

Version: 1.4

Revised: November 2019

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1. Introduction

This quick guide is meant for experienced users. Please review the full "Instruction for use" carefully before using the kit. The assay is comprised of one reference assay and one mutation assay. The reference assay is used for quantification of the total amplifiable DNA input, whereas the existence of the *KIT* D816V mutation is identified by a positive signal in the mutation specific assay. Furthermore, an internal control is present in both mixes to verify amplification in mutation negative analyses.

2. Content

The kit comprises 20 or 50 reactions of reference assay reagents, triplicate mutation-specific assay reagents and AmpliQueen™ Master mix. All reagents needed for the analysis of extracted, genomic DNA (gDNA) are included and ready to be dispensed into real-time PCR tubes or plates.

3. Storage and Stability

The unopened product is stable at -20˚C for a minimum of 15 months, but no longer than the expiry date. **Important:** Keep frozen until use and thaw at room temperature. Avoid repeated freeze/thaw cycles.

4. DNA extraction

- Use gDNA from liquid biopsies or any other suitable biopsy material
- gDNA can be extracted using any valid gDNA extraction kit
- Follow the instructions for gDNA extraction recommended by the kit supplier
- Determine the quantity and quality of gDNA prior to real-time PCR. Do not use gDNA of a low quality
- Use 1-40 ng/µL gDNA per reaction/tube

5. Kit preparation

Thaw all reagents and spin down.

6. Setup

- Prepare reactions by adding the components according to Table 1. For each patient sample setup, the reference and mutant specific mixtures in separate qPCR-tubes or wells

Table 1: PlentiPlex® Dispense Ready mix

Components	Vol./reaction
Reference mixture	7.5 µL
Mutation specific mixture (triplicates)	7.5 µL
Master mix	12.5 µL
Patient DNA (1-40 ng/µL)	5.0 µL

- Gently mix with a pipette, seal vial and spin down
- Perform real-time PCR using the program shown in Table 2

Protocol	Temperature	Time	Ramping (°C/s)	Cycles	Data (channel)
Hold	95˚C	2 min		1	-
Cycling	94˚C	15 sec	<2	45	FAM™/SYBR® (470 nm/510 nm) HEX™/VIC™/TET™ (538 nm/551 nm) Measure fluorescence intensity at the end of each cycle
	60˚C	40 sec	≤1.5		

Table 2. PlentiPlex® Mastocytosis PCR protocol

7. NOTES

- All test components should be stored as described in the "Instructions for use" (storage section)
- Do not mix reagents from different lots
- Always spin down before opening the lids
- For each sample setup the mutant specific assay in triplicates together with corresponding reference assay in separate vials/wells

8. DATA ANALYSIS

- Correct for "baseline drift" before setting the threshold. Please refer to the "Instructions for use" for details
- Set the threshold for PentaGreen™ at 10% of the fluorescence signal of the reference assay at cycle 45. Add any significant assay baseline fluorescence at cycle 20 to the threshold value
- Samples giving no signal for neither the assay (PentaGreen™) nor the internal control (PentaYellow™) are invalid. Setup a new real-time PCR for these
- Verify reference Ct according to Table 3.

Ct for reference	Quality	Comments
Ct, reference <23	Not valid	The amount of input DNA is too high which might affect the assay. The analysis should be repeated with lower input of DNA
23≤ Ct, reference ≤32	Optimal	The amount of input DNA is valid for mutation analysis. Please note that the closer the reference Ct is to 23, the higher the number of potential *KIT* D816V-mutated gDNA templates are analysed.
Ct, reference >32	Not optimal	The amount of input DNA is low. If the sample is negative for the mutation, the analysis should be repeated with higher amount of input DNA if possible since a reduced number of copies of gDNA are analyzed leading to a substantial risk of false negatives.

Table 3: Reference Ct validation

- Calculate ΔCt for each of the triplicate mutation assays having a Ct value equal to or lower than 44.

$$\Delta Ct = Ct_{\text{Mutation assay}} - Ct_{\text{Reference assay}}$$

- A sample is positive for the mutation if the reference Ct is valid (Table 3) and the Ct of the mutation assay ≤ 44 and if the ΔCt is equal to or below 20 for **at least 2 of the 3 mutation assays** (Table 4).

Ct and ΔCt for assay	Conclusion	Comments
Ct ≤ 44 and ΔCt ≤ 20 in min. 2 of 3 assays	**Positive**	The sample is positive for the mutation if Ct ≤ 44 and ΔCt ≤ 20 for at least 2 out of the 3 assays and the reference is valid
Ct > 44 (or no Ct) or ΔCt > 20 in 2 of 3 assays	**Negative**	The sample is negative for the mutation if Ct > 44 or ΔCt > 20 for 2 out of the 3 assays and the reference is valid

Table 4: Determination of *KIT* D816V mutational status based on Ct and mean ΔCt values.

9. TROUBLESHOOTING

This short troubleshooting guide may assist in solving most frequent encountered problems that can occur. Please refer to the "Instructions for use" for further troubleshooting.

- If no signal in neither PentaYellow nor PentaGreen is present, no amplification has taken place indicating that there is no amplifyable DNA in the patient sample (e.g. degraded DNA or contamination with PCR inhibitors). Check DNA quality and if possible, repeat PCR with higher DNA quality/input
- Too low Ct value in PentaGreen for the reference indicates that the amount of DNA is too high. If possible, repeat PCR with lower DNA input
- High Ct value in PentaGreen for the reference indicates that the amount of DNA is low. If possible, repeat PCR with higher DNA input
- Fluorescence drift could result from either sample or instrument instabilities or air bubbles