

# PlentiPlex™ Mastocytosis

Made by PentaBase

## INSTRUCTIONS FOR USE

PlentiPlex™ assay for sensitive detection of the *KIT* D816V mutation



#Cat. No.: 7021-7024

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

Version: 1.2

Revised: November 2019

PentaBase ApS | Lumbyvej 19G | DK-5000 Odense | Denmark

---

## TABLE OF CONTENTS

---

<b>TABLE OF CONTENTS</b> .....	<b>1</b>
<b>1. INTENDED USE</b> .....	<b>3</b>
<b>2. SUMMARY AND EXPLANATION OF THE ASSAYS</b> .....	<b>3</b>
<b>3. TECHNOLOGY AND REAGENTS</b> .....	<b>3</b>
3.1 <i>HydrolyEasy™ Probes</i> .....	4
3.2 <i>SuPrimers™</i> .....	4
3.3 <i>BaseBlockers™</i> .....	4
<b>4. ASSAY FORMAT AND DESIGN</b> .....	<b>4</b>
4.1 <i>Format</i> .....	4
4.2 <i>Internal control</i> .....	5
4.3 <i>Reference assay</i> .....	5
4.4 <i>Mutation assay</i> .....	5
4.5 <i>Equipment and reagents not supplied with PlentiPlex™ Mastocytosis</i> .....	6
<b>5. SAFETY, SHIPMENT AND STORAGE</b> .....	<b>6</b>
5.1 <i>Precautions</i> .....	6
5.2 <i>Shipment</i> .....	7
5.3 <i>Storage</i> .....	7
<b>6. SPECIMENS</b> .....	<b>7</b>
6.1 <i>Recommended procedure for extraction of gDNA from whole blood</i> .....	7
<b>7. PLENTIplex™ MASTOCYTOSIS PROTOCOL</b> .....	<b>7</b>
7.1 <i>Ready-to-use</i> .....	7
7.2 <i>Dispense Ready</i> .....	8
<b>8. DATA ANALYSIS</b> .....	<b>8</b>
8.1 <i>Adjusting the baseline</i> .....	8
8.2 <i>Determining the mutational status</i> .....	9
8.3 <i>Internal control analysis</i> .....	10
<b>9. TROUBLESHOOTING</b> .....	<b>10</b>
<b>10. REFERENCES</b> .....	<b>10</b>
<b>11. MANUFACTURER AND DISTRIBUTORS</b> .....	<b>10</b>
11.1 <i>Manufacturer</i> .....	10
11.1 <i>Technical assistance</i> .....	11
11.2 <i>Date of revision</i> .....	11

<b>12. ADDITIONAL INFORMATION .....</b>	<b>11</b>
<b>13. ADDITIONAL INFORMATION .....</b>	<b>11</b>
<b>14. NOTES.....</b>	<b>12</b>

## Δ IMPORTANT:

Please read these instructions carefully before using the PlentiPlex™ Mastocytosis assay. It is recommended to save the “Instructions for use” for future use. Purchasers of PlentiPlex™ Mastocytosis assay are only granted the right of use, but no general licensing or patent rights.

### 1. INTENDED USE

PlentiPlex™ Mastocytosis is intended for in vitro detection of the aspartic acid to valine mutation in codon 816 of the KIT proto-oncogene receptor tyrosine kinase (KIT D816V, c.2447A>T) in human genomic DNA (gDNA) purified from blood samples. The test will provide an assessment of the presence of the examined mutation constituting down to 0.002 % of a human gDNA sample. PlentiPlex™ Mastocytosis is to be used by trained laboratory personnel in a professional laboratory environment and the test is provided with all necessary reagents for amplification of the human *KIT* D816V allele by real-time PCR.

PlentiPlex™ Mastocytosis is intended for research use only. For diagnostic purposes, please refer the PlentiPlex™ Mastocytosis CE IVD (#Cat. No.: 7030-7033).

### 2. SUMMARY AND EXPLANATION OF THE ASSAYS

Mastocytosis is a disorder characterized by growth and accumulation of neoplastic mast cells within various organs and most commonly the skin. The WHO criteria for diagnosis of systemic mastocytosis include identification of at least one major criterion as well as one minor criterion or identification of at least three minor criteria. The major systemic mastocytosis criterion is multifocal dense infiltrates of mast cells ( $\geq 15$  mast cells in aggregates) in bone marrow biopsies and/or in sections of other extracutaneous organ(s). The three minor criteria are: 1) more than 25% of all mast cells are atypical cells (type I or type II) on bone marrow smears or are spindle-shaped in mast cell infiltrates detected on sections of visceral organs, 2) *KIT* point mutation at codon 816 in the bone marrow or another extracutaneous organ, 3) mast cells in bone marrow or blood or another extracutaneous organ exhibit CD2 and/or CD25, and 4) baseline serum tryptase level  $>20$  ng/mL (in case of an unrelated myeloid neoplasm, item 4 is not valid as a systemic mastocytosis criterion) (Valent et al. 2016). Although the fraction of cells carrying the *KIT* D816V mutation is often very low ( $<0.1\%$  mutated allele), presence of the mutated allele is well correlated with mastocytosis.

PlentiPlex™ Mastocytosis is based on PentaBase’s highly sensitive DNA technology and can, if provided with sufficient input of DNA of sufficient quality, detect 0.01% of *KIT* D816V mutated gDNA in a background of wild type gDNA.

KIT mutation detected with PlentiPlex™ Mastocytosis			
Assay	CDS mutation	Amino acid substitution	Cosmic ID
KIT D816V	c.2447A>T	p.Asp816Val (D816V)	COSM1314

Table 1. List of investigated mutations

### 3. TECHNOLOGY AND REAGENTS

PlentiPlex™ Mastocytosis combines allele-specific PCR with PentaBase’s novel and selective technologies comprising: 1) HydrolEasy™ probes, 2) SuPrimers™ for specific and sensitive amplification, and 3) BaseBlockers™. The technology is applicable on standard real-time equipment using standard procedures. Pentabases are synthetic DNA analogues comprising a flat heteroaromatic, hydrophobic molecule and a linker. They are inserted into the oligonucleotides at fixed positions during synthesis. PlentiPlex™ Mastocytosis contains both standard oligonucleotides and pentabase-modified oligonucleotides

(HydrolyEasy™ probes, SuPrimers™, and BaseBlockers™). Using PlentiPlex™ Mastocytosis, somatic mutations can be detected quickly (in less than one and a half hour), sensitively (5-400 ng gDNA input per well/vial) and selectively (0.002% mutation in a wild type background of gDNA), by real-time PCR analysis.

### 3.1 HYDROLEASY™ PROBES

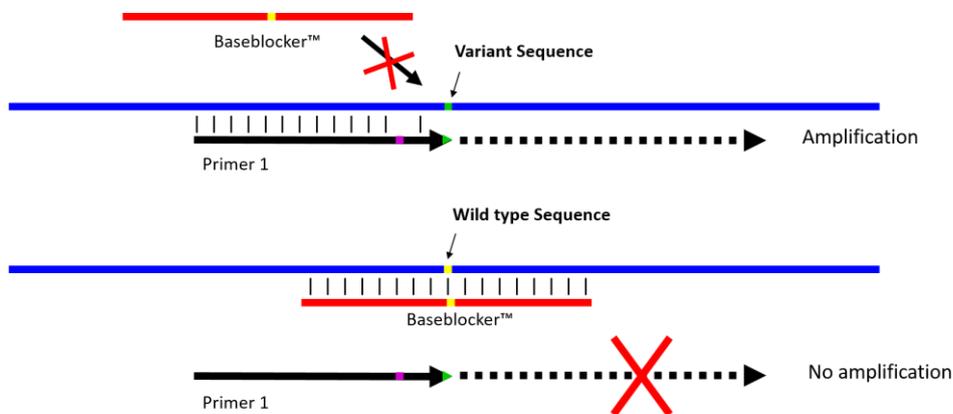
A **HydrolyEasy™** probe is similar to a standard hydrolysis probe (also referred to as a TaqMan™ probe) labeled with a fluorophore at the 5' end, a quencher at the 3' end, but with the addition of pentabases giving the probe a significantly improved signal-to-noise ratio, higher specificity and higher sensitivity compared to conventional hydrolysis probes. HydrolyEasy™ probes in PlentiPlex™ Mastocytosis assays are labeled with PentaGreen™ ( $\lambda_{\text{abs.}}$  495 nm and  $\lambda_{\text{Em.}}$  516 nm, detected on the same channel as FAM™) in combination with Green Quencher™, or as PentaYellow™ ( $\lambda_{\text{abs.}}$  533 nm and  $\lambda_{\text{Em.}}$  557 nm, detected on the same channel as HEX™, VIC™, TET™) in combination with Yellow Quencher™.

### 3.2 SUPRIMERS™

**SuPrimers™** are standard DNA primers modified with one or more pentabases. The pentabases provide increased specificity and sensitivity and reduce primer-dimer formation.

### 3.3 BASEBLOCKERS™

**BaseBlockers™** are DNA sequences modified with several pentabases, allowing for the specific and strong binding to a target sequence. In PlentiPlex™ Mastocytosis, the BaseBlockers™ are designed to bind to wild type gDNA targets, suppressing false positive signals from the wild type templates and ensuring high specificity and robustness of the assay. Along with SuPrimers™, the BaseBlockers™ minimize or eliminate the risk of false positive signals. The BaseBlocker™ principle is illustrated below.



**Figure 1:** Illustration of how BaseBlockers™ function in PlentiPlex™ assays. A BaseBlocker™ binds to and blocks the wild type template from being amplified. In contrast, the BaseBlocker™ does not inhibit amplification of a template with a single nucleotide mutation and the result is a selective amplification of mutated gDNA in a wild type background.

## 4. ASSAY FORMAT AND DESIGN

### 4.1 FORMAT

PlentiPlex™ Mastocytosis is supplied in either a "Ready-To-Use" or "Dispense Ready" version. PlentiPlex™ Mastocytosis Ready-to-use assays are provided in either 1, 12 or 60 reactions in pre-aliquoted PCR strips (Table 2) while Dispense Ready assays are provided in 20 or 50 reactions (Table 3).

**PlentiPlex™ Mastocytosis contains the following reagents:**

*Reference assay*

- Reference assay primer/probe mix (labeled with PentaGreen™, for detection on green (FAM™) channel)
- Internal control primer/probe mix (labeled with PentaYellow™, for detection on yellow (HEX™) channel)
- AmpliQueen™ Master mix

*Mutation assay*

- Mutation assay primer/probe/BaseBlocker™ mix (labeled with PentaGreen™, for detection on green (FAM™) channel)
- Internal control primer/probe mix (labeled with PentaYellow™, for detection on yellow (HEX™) channel)
- AmpliQueen™ Master mix

Mastocytosis KIT D816V Assay					
Strip #	Gene	Tube #	Content	Mutation	Corresponding reference
IM1	KIT	A	KIT Reference 1		
		B	KIT Simplex 1	D816V	KIT Reference 1
		C	KIT simplex 1	D816V	KIT Reference 1
		D	KIT simplex 1	D816V	KIT Reference 1

**Table 2.** PlentiPlex™ Mastocytosis Ready-to-use assay components. Each tube contains 20 µL in total (7,5 µL primer/probe-mix and 12,5 µL master mix).

Mastocytosis KIT D816V Assay						
Gene	Tube #	Content	Mutations	Corresponding reference	Volume 20x	Volume 50x
KIT	1	KIT Reference 1			150 µL	375 µL
	2	KIT Simplex 1	D816V	KIT Reference 1	450 µL	1125 µL
	3	Master mix			1000 µL	2500 µL

**Table 3.** PlentiPlex™ Mastocytosis Dispense Ready assay components. Each tube contains reagents for either 20 or 50 reactions.

**4.2 INTERNAL CONTROL**

An internal control assay is included in the primer-probe mixes of the assay and comprise a HydrolEasy™ probe labeled with PentaYellow™ (measured on the same fluorescence channel as HEX™, VIC® or TET™) and a primer set. The internal control assay is used to assess whether an amplification has taken place in reactions with negative signal from the PentaGreen™ labeled assay in the same reaction. The primers in the control assay are designed to be inefficient and are located outside the area of all frequently known mutations. In this way, the internal control assay will have as little impact on the effectiveness of the reference and the mutation-specific assays as possible. The signal from the internal control assay may be affected by positive amplification in the reference and mutation-specific assays. See section 8 “Data analysis” for more details.

**4.3 REFERENCE ASSAY**

The reference assay targets a genomic region with no known sequence variations and is used to assess the amount of amplifiable DNA in the sample. The reference assay contains a HydrolEasy™ probe labeled with PentaGreen™ (measured on the FAM™ channel), a mutation-independent primer set and an internal control assay. The reference assay runs in its own tube or well. The fluorescence signal of the reference assay is used for calculating the threshold value which is again used to determine the cycle threshold (Ct) of the assay of interest.

**4.4 MUTATION ASSAY**

The mutation assay (Table 2-3) targets the genomic region containing the *KIT* D816V mutation (c.2447A>T) and is used to determine the presence of the mutation in a sample (see section 8 “Data Analysis” for more details). The mutation assay contains a HydrolEasy™ probe labelled with PentaGreen™ (measured on the FAM™ channel), a BaseBlocker™ (to reduce or

eliminate non-specific amplification of wild type), a mutation-specific primer set, and an internal control assay. The mutation-assay is optimized to the conditions specified in section 7 and it is therefore important that these are followed to avoid misleading results. The Ct values of the mutation assay and the corresponding reference are used to determine whether a sample is positive or negative for a given mutation. See section 8 “Data Analysis” for more details.

#### 4.5 EQUIPMENT AND REAGENTS NOT SUPPLIED WITH PLENTIplex™ MASTOCYTOSIS

The use of PlentiPlex™ Mastocytosis will require the following equipment and consumables:

- Template DNA (extracted mutant gDNA)
- Real-Time PCR instrument\*
- Plastic products (tubes/plates) that are compatible with the instrument^
- Dedicated pipettes and tips for preparing PCR mixes
- Dedicated pipettes and tips for addition of DNA sample
- Centrifuge for spinning tubes/plates
- Nuclease-free H<sub>2</sub>O (sterile)

\* PlentiPlex™ Mastocytosis has been validated on the following real-time PCR instruments: Rotorgene (Qiagen), MyGo Pro (IT-IS Life), and Mic qPCR cycler (Bio Molecular Systems). We recommend that one of these systems are used, but other instruments are likely applicable.

^ PlentiPlex™ Mastocytosis ready-to-use assays include PCR strips for use in analysis (can be provided in either 0.1 mL or 0.2 mL strips)

## 5. SAFETY, SHIPMENT AND STORAGE

---

General laboratory precautions should be taken. PlentiPlex™ Mastocytosis should only be used by personnel who has been trained in the appropriate techniques. All chemicals and biological material should be considered as potentially hazardous. When working with the assay suitable personal protective equipment (lab-coat, disposable gloves and safety glasses) should be used. It is recommended that all work is carried out in appropriate facilities. All waste should be disposed as clinical waste.

### 5.1 PRECAUTIONS

The following precautions should be taken when working with PlentiPlex™ Mastocytosis:

- The assay is only intended for *in vitro* diagnostics
- PlentiPlex™ Mastocytosis is not intended for sole diagnosing of mastocytosis, but only as a supplement for other diagnostic factors
- The mutational status determined by PlentiPlex™ Mastocytosis should always be considered alongside other disease factors when making treatment decisions
- Avoid several freeze/thaw cycles of the reagents as this might impair the performance of PlentiPlex™ Mastocytosis assays. Limit to a maximum of eight times
- Verify eligibility of the DNA samples as DNA samples can be non-homogeneous and of varying quality, which might affect the analysis
- The delivered reagents should not be diluted further. Further dilution can cause loss of performance and increase the risk of false negative and false positive results
- Use the specified volumes. It is not recommended to reduce the specified volumes as the results can be affected
- No reagents should be substituted by others if the optimal performance should be maintained
- It is recommended to use one of the platforms, validated to ensure full PlentiPlex™ Mastocytosis performance. For more information, see section 4.5 “Equipment and Reagents not supplied with PlentiPlex™ Mastocytosis”

- Due to the presence of HydrolEasy™ probes, assays should be protected from light
- Use extreme caution not to contaminate reagents and samples. It is recommended to separate preparation of PCR mixes and gDNA addition. Dedicated pipettes should be used, and it is recommended to have separate areas for sample preparation and PCR running
- PCR tubes should not be opened after completing the PCR program
- All used instruments and equipment should be calibrated and meet their original specifications

## 5.2 SHIPMENT

PlentiPlex™ Mastocytosis Ready-to-use assays are shipped on dry ice while Dispense Ready assays are shipped on either dry ice or blue ice. If the PlentiPlex™ Mastocytosis packaging has been opened during transport or if the products are not frozen upon arrival, please contact your local distributor or PentaBase ApS (see section 11 “Manufacturer and Distributors”). Please also contact your local distributor or PentaBase ApS if the shipment is missing a certificate of analysis, reagents or a “Quick Guide”.

## 5.3 STORAGE

PlentiPlex™ Mastocytosis assays should after arrival immediately be stored at maximum -15°C. Repeated freeze/thaw cycles should be avoided. If the assays are stored under the recommended conditions, they should be stable until the date stated.

## 6. SPECIMENS

---

Specimens should be human genomic DNA extracted from whole blood samples or bone marrow. The samples should be collected and stored after standard pathology methodology to ensure optimal quality. Extracted gDNA should be stored at maximum -18°C until use.

### 6.1 RECOMMENDED PROCEDURE FOR EXTRACTION OF gDNA FROM WHOLE BLOOD

DNA isolation should be performed using a kit designed, approved and quality controlled for extraction of genomic DNA from whole blood according to the manufacturer’s protocol.

## 7. PLENTIplex™ MASTOCYTOSIS PROTOCOL

---

Before using the assay, it is recommended to carefully read this instructions for use. PlentiPlex™ Mastocytosis is supplied in either a "Ready-to-use" or "Dispense Ready" version. When using PlentiPlex™ Mastocytosis Dispense Ready assays, it is recommended to collect samples in larger batches for most effective use of reagents and to avoid repeated freeze/thaw cycles and waste. For each sample, a reference assay must be included in the mutation analysis (Table 2-3). These should be analyzed in the same PCR run to ensure minimal variation.

### 7.1 READY-TO-USE

1. Thaw the reaction mixtures and spin down
2. Add 5 µL extracted gDNA (1-40 ng/µL) to the reference and mutation assays. It is recommended to include a no template control (NTC) in each run. Add sterile water instead of gDNA.
3. Close lids and spin down
4. Place the strips into the instrument and run the protocol described in Table 4
5. Analyze the samples in accordance with the analysis rules. For more information, see section 8 “Data analysis”

Protocol	Temperature (°C)	Time (s)	Ramping (°C/s)	Number of cycles	Data (channel)
Hold	95	120	-	-	-
Cycling	94	15	≤2	45	FAM™/SYBR® (470 nm/510 nm) HEX™/VIC™/TET™ (538 nm/551 nm)
	60	40	≤1.5		Measure fluorescence intensity at the end of each cycle

**Table 4.** PlentiPlex™ Mastocytosis Real-time PCR protocol

## 7.2 DISPENSE READY

1. Run the mutation specific assay in triplicates
2. Thaw the reaction mixtures, mix and spin down
3. Add 12.5 µL master mix to all tubes/wells
4. Add 7.5 µL of reference mix to one tube/well per patient and mix carefully by pipetting
5. Add 7.5 µL of mutant mix to three tubes/wells per patient and mix carefully by pipetting
6. Add 5 µL extracted gDNA (1-40 ng/µL) from each sample to the mutation assays and the corresponding reference. Mix carefully by pipetting. It is recommended to include a NTC in each run. Add sterile water to the NTC instead of gDNA.
7. Seal all tubes/wells and spin down. Make sure that there are no bobbles in the solutions
8. Place tubes or plate in the instrument and run the protocol as described in Table 4
9. Analyze the samples in accordance with the analysis rules. For more information, see section 8 “Data analysis”

## 8. DATA ANALYSIS

When using PlentiPlex™ Mastocytosis real-time PCR assays, determining the cycle threshold (Ct) is a central part of the data analysis procedure. Ct is defined as the cycle in which the fluorescence signal of a given assay exceeds the threshold value. The threshold is set to 10% of the reference fluorescence signal at cycle 45 (Figure 2). **Important:** The following data analysis procedure is based on studies using Rotor-Gene Q, MyGo Pro and Mic real-time PCR instruments. The Ct and delta Ct cutoff values have been set to provide a satisfactory balance between obtaining false positive and false negative results using these instruments. It is however in any case recommended to perform a validation of the real-time PCR instrument to be used with the PlentiPlex™ Mastocytosis assay including analysis of known *KIT* D816V mutation positive and negative samples.

### 8.1 ADJUSTING THE BASELINE

Before setting the threshold value and calculating the Ct values, it is important that any baseline “drift” or fluctuation is corrected so that the baseline or background fluorescence is as close to zero as possible. Different instrument manufacturers use different approaches to adjust the baseline. These include slope correction, curve fitting, setting a baseline cycling interval and ignoring the first cycles in the run. Please refer to the instrument-specific guidelines for specific instructions when available.

**IMPORTANT!** In cases where it is not possible to adjust the baseline fluorescence to zero, the value of baseline fluorescence at cycle 20 should be added to the threshold value calculated by taking 10 % of the reference signal at cycle 45. An example of this is shown in Table 5.

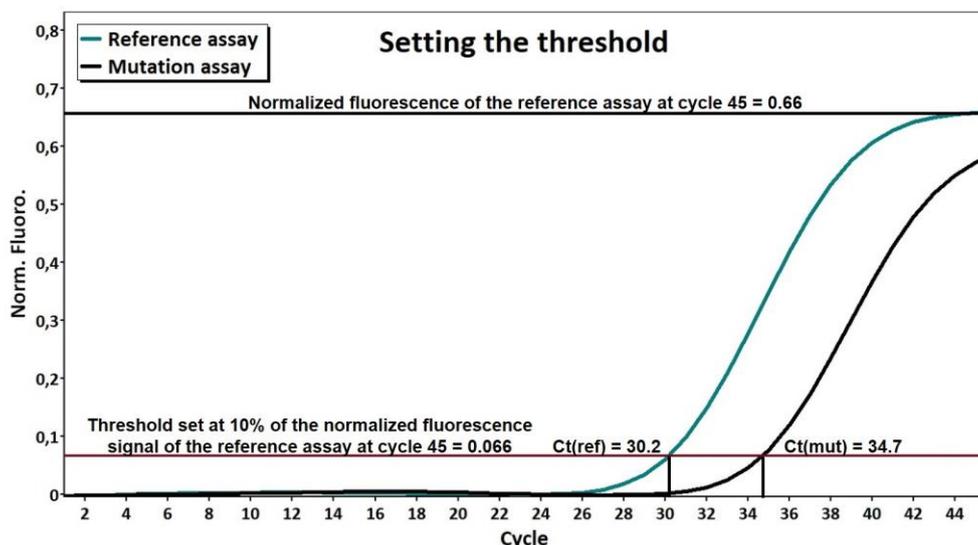
Reference fluorescence at cycle 45	10% of reference fluorescence at cycle 45	Assay baseline/background fluorescence at cycle 20	Threshold value
3	0.3	0	0.3
3	0.3	0.2	0.5

**Table 5.** Setting the threshold.

## 8.2 DETERMINING THE MUTATIONAL STATUS

Use the following protocol to determine the mutational status:

1. Analyze the mutation assay against the corresponding reference for one sample at a time
2. Correct for "baseline drift" before setting the threshold:
  - Use slope correction/curve fitting when possible and/or define the baseline or background cycle interval to be between cycle 15 and cycle 20
3. Set the threshold at 10% of the reference signal strength at cycle 45 (Figure 2). Add any significant baseline fluorescence at cycle 20 to the threshold value (Table 5)



**Figure 2:** Setting the threshold. Read the fluorescence value for the reference assay at cycle 45 and set the threshold at 10% of this value. This setting for the threshold is now used for the analysis of the corresponding mutation assay. In the shown example, Ct(reference)=30.2 and Ct(mutation)=34.7.

4. If NTC samples have been included in the run, verify that no signal is seen before Ct = 38 for the reference and Ct = 44 for mutation analyses. A positive signal in the NTC before these limits indicates contamination, which may influence the results. Data should not be used if the NTC control is positive
5. Read the Ct value for the reference assay and validate that the reference sample is suitable for analysis, cf. Table 6.

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 number of potential <i>KIT</i> D816V-mutated gDNA templates are analyzed.
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 6.** Acceptable Ct values for the reference assay.

6. Calculate  $\Delta Ct$  for each of the mutations having a Ct value equal to or lower than 44.

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

7. A sample is positive for the mutation if the reference Ct is valid (Table 6) and the Ct of the mutation assay  $\leq 44$  and if the  $\Delta Ct$  is equal to or below 20 for **at least 2 of the 3 assays** (Table 7).

Ct and $\Delta$ Ct for assay	Conclusion	Comments
Ct $\leq$ 44 and $\Delta$ Ct $\leq$ 20 in min. 2 of 3 assays	<b>Positive</b>	The sample is positive for the mutation if Ct $\leq$ 44 and $\Delta$ Ct $\leq$ 20 for at least 2 out of the 3 assays and the reference is valid
Ct > 44 (or no Ct) or $\Delta$ Ct > 20 in 2 of 3 assays	<b>Negative</b>	The sample is negative for the mutation if Ct > 44 or $\Delta$ Ct > 20 for 2 out of the 3 assays and the reference is valid

**Table 7.** Determination of *KIT* D816V mutational status based on Ct and  $\Delta$ Ct values.

### 8.3 INTERNAL CONTROL ANALYSIS

In reactions with no or late amplification by the PentaGreen™ labeled assay, it should be validated that template has been added and/or amplification has taken place by examining the fluorescence from the internal control assay (yellow channel). To set the threshold for the internal control assay, select the yellow channel and repeat steps 1-3 in section 8.3. **Note:** The internal control assay contains suboptimal primer concentrations and amplification may be inhibited by amplification by the PentaGreen™ labeled assay in the same reaction. Thus, the Ct value of the internal control assay is only indicative of the amount of template added to the reaction and cannot be used for precise quantification of DNA.

## 9. TROUBLESHOOTING

The troubleshooting guide shown in Table 8 below covers some of the most frequent questions and problems that can occur with the use of PlentiPlex™ Mastocytosis assay and how they might be solved.

Problem	Solution
NTC signal	The assay is contaminated. Find the cause of contamination by checking all sources such as water, pipettes or facilities. If the contamination can't be located contact Pentabase ApS. For contact details, see section 11 "Manufacturer and Distributors"
No internal control signal (PentaYellow™)	There is no lower threshold for the Yellow channel. No internal control signal is only a problem if there is no signal on the green channel either. This indicates that no amplification has occurred. This might be due to low amount or low quality of gDNA or the presence of PCR inhibitors. Repeat the PCR with higher gDNA quality. If there is a signal on the reference assay (in the green channel) with Ct<30 but no signal in the internal control of the mutation assays, then try to dilute the gDNA five times and repeat the PCR
No reference signal (PentaGreen™)	No reference signal indicates that a low amount or low quality gDNA has been used. If there are no signal (before Ct = 44) in the mutation-specific assays either, the purification of gDNA should be re-done. If a signal is observed in some of the mutation-specific assays or in other reference assays with the specific sample, the analysis could be re-run using present extraction of gDNA
No signal from mutation specific assay (PentaGreen™)	Check that there is signal for the internal control (yellow channel). If there is a signal this sample does not comprise the specific mutation
Ct(reference) < 23	The amount of input gDNA is too high. This can affect the performance of PlentiPlex™ Mastocytosis. Repeat the PCR with lower input of gDNA if possible
Ct(reference) > 32	The amount of input gDNA is too low. If possible, repeat the PCR with higher input of gDNA. If the mutation-specific analysis is positive, the sample is most likely mutated

**Table 8.** Troubleshooting

## 10. REFERENCES

Valent, P., Akin, C., and Metcalfe, D.D. (2017). Mastocytosis: 2016 updated WHO classification and novel emerging treatment concepts. *Blood*, 129(11): 1420-1427.

## 11. MANUFACTURER AND DISTRIBUTORS

### 11.1 MANUFACTURER

	Pentabase ApS Lumbyvej 19G 5000 Odense C, Denmark
-------------------------------------------------------------------------------------	---------------------------------------------------------

### 11.1 TECHNICAL ASSISTANCE

For technical assistance in Denmark, contact PentaBase ApS:

[www.pentabase.com](http://www.pentabase.com)

[support@pentabase.com](mailto:support@pentabase.com)

Phone: +45 3696 9496

For technical assistance in all other countries, contact your local distributor. A complete list of distributors is available at

[www.pentabase.com](http://www.pentabase.com).

### 11.2 DATE OF REVISION

PlentiPlex™ Mastocytosis protocol was revised in November 2019.

### Change history

Version No.	Effective Date	Significant Changes	Previous version
1.02	08.05.2018	New Procedure	N/A
1.10	15.04.2019	Changed the maximum gDNA concentration from 80 to 40 ng/μl and corrected table 6 Ct cut off value for the reference assay from Ct≥21 to Ct≥23	1.02
1.2	06.11.2019	Updated sections 1,2, 6 and 8	1.1

## 12. ADDITIONAL INFORMATION

Assay	PlentiPlex™ Ready-to-use	Assay #	Catalogue # 12; 60 reactions	PlentiPlex™ Dispense Ready	Catalogue # 20; 50 reactions
Mastocytosis	KIT D816V Ready-to-use (RUO)	IM2	7021-7022	KIT D816V Dispense Ready (RUO)	7023-7024

## 13. ADDITIONAL INFORMATION

TaqMan™ is a trademark of Roche. 5-FAM™, VIC™, TET™ and HEX™ are trademarks and registered trademarks of Applied Biosystems Corporation or its subsidiaries in the U.S. and certain other countries. PlentiPlex™, HydrolEasy™, SuPrimers™ and BaseBlockers™ are all trademarks belonging to PentaBase ApS. Products or parts of it must not be resold or transferred without PentaBase ApS acceptance. PentaBase ApS takes certain reservation for changes. PentaBase ApS disclaim all responsibility for any errors that may appear in this Instructions for use. Furthermore, PentaBase ApS disclaim all responsibility for misinterpretation that can occur by using this product.

© Copyright 2018. PentaBase ApS reserves all rights associated with this product.

PentaBase ApS

Lumbyvej 19G

5000 Odense C

Denmark

[www.pentabase.com](http://www.pentabase.com)

[support@pentabase.com](mailto:support@pentabase.com)

Phone: +45 3696 9496

