

Plenti[®]lex[™] MYD88

Made by PentaBase

READY-TO-USE QUICK GUIDE

Plenti[®]lex[®] Assay for Sensitive Detection of the MYD88 L265P Mutation



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

1. INTRODUCTION

This quick guide is meant for experienced users. Please review the full “Instructions 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 MYD88 L265P 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 12 or 60 strips suitable for most standard 96-well plate format real-time PCR instruments. All reagents needed for the analysis of extracted genomic DNA are pre-loaded into the strips.

3. STORAGE AND STABILITY

The unopened product is stable at -20°C for a minimum of 9 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 1-10 ng/μL gDNA per reaction/tube

5. KIT PREPARATION

Thaw one strip per sample per analysis (3 strips if running triplicates) and spin down, before removing lids gently.

6. SETUP

1. Add 5 μL extracted patient DNA (1-10 ng/μL) per tube
2. Gently mix with a pipette, seal vial and spin down
3. Perform real-time PCR using the program shown in Table 1

Table 1. PlentiPlex® MYD88 L265P PCR protocol

Protocol	Temperature	Time	Cycles	Data (channel)
Hold	95°C	2 min	1	-
Cycling	94°C	15 sec	45	FAM™/SYBR® (470 nm/510 nm)
				HEX™/VIC™/TET™ (538 nm/551 nm)
	57°C	10 sec		Measure fluorescence intensity during annealing (57°C)
	72°C	15 sec		

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 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 2.

Table 2: Reference Ct validation

Ct for reference	Quality	Comments
Ct, reference <25	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
25 ≤ Ct, reference ≤ 31	Optimal	The amount of input DNA is optimal for the results of the mutations analysis
31 < Ct, reference ≤ 36	Borderline	The amount of input DNA is lower than recommended. The sensitivity is affected hereby. The analysis should if negative be repeated with higher amount of input DNA if possible
Ct, reference >36	Not valid	The amount of input DNA is too low. The analysis should be repeated with higher amount of input DNA

- Read the Ct value for the mutation assay
- Calculate the ΔCt for the mutation assay when the Ct value is equal to or lower than 39. ΔCt is calculated as the Ct value of the mutation assay(s) subtracted the Ct value of the reference assay

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

- Use the calculated ΔCt value to evaluate the mutation status according to Table 3

Table 3: Evaluation of mutation status

ΔCt for Simplex	Conclusion	Comments
ΔCt ≤ 9	Positive	If ΔCt ≤ 9 and the mutation assay Ct is ≤ 39, the sample is positive for the mutation
ΔCt > 9	Negative	If ΔCt > 9 and the mutation assay Ct is ≤ 39, the sample is negative for the mutation

- For mutation negative samples, it should be validated that amplification has taken place by examining the fluorescence from the internal control assay (yellow channel). An amplification curve (in contrast to a flat line) is evidence that amplification has taken place. Although the Ct value of the internal control assay will reflect the amount of DNA in the sample, there are no specific requirements to the signals in the yellow channel.
- Samples giving no signal for neither the assay (PentaGreen™) nor the internal control (PentaYellow™) are invalid. Setup a new real-time PCR for these

9. TROUBLESHOOTING

This short troubleshooting guide may assist in solving most frequent encountered problems that can occur. Please refer to “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 amplifiable 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