



SENSISCREEN® FFPE READY-TO-USE CE IVD QUICK GUIDE
SensiScreen® assays for sensitive detection and identification of mutations in cancer



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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 or more reference assays and one or more mutation assays. The reference assay is used for determination of total amplifiable DNA input and thus validity of the sample, whereas the existence of mutation(s) is identified by a positive signal in the mutation specific assay(s). Furthermore, an internal control is present in all the mixes to verify the PCR amplification has taken place.

2. CONTENT

The kit comprises 12 or 60 x 0.2 mL (regular profile) or 0.1 mL (low profile) strips suitable for most standard 96-well plate format real-time PCR instruments. All reagents needed for the analysis of extracted human DNA are included and 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 FFPE, fresh frozen or any other suitable biopsy material
- Genomic DNA (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 5-50 ng gDNA per reaction/tube

5. KIT PREPARATION

Thaw one strip per sample to be analysed and spin down before removing lids gently

6. SETUP

1. Add 5 µL extracted patient DNA (1-10 ng/µL) pr. tube
2. Gently mix with a pipette, re-seal tubes and spin down
3. Perform the real-time PCR using the protocol described in Table 1

Table 1: SensiScreen® 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)
	60°C	60 sec		HEX™/VIC™/TET™ (538 nm/551 nm)
				Measure fluorescence intensity at the end of each cycle

7. NOTES

- All test components should be stored as described in the “Instructions for use” (storage section)
- Do not mix reagents from different lots
- Use one full strip per patient sample. Never mix patient material in one strip
- Always spin down before removing the lids
- Be careful not to damage lids when opening/closing
- Add patient DNA into all tubes in a strip
- Make sure that all tubes are properly sealed and spin down the strips to remove air bubbles
- Tube number one (closest to strip annotation) comprises the (first) reference assay
- Make sure that all tubes are properly sealed and spin down the strips vigorously to remove air bubbles
- Tube number one is marked with strip annotation and comprise a reference assay

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
- Read the Ct value for the reference assay and validate that the reference sample is suitable for analysis 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(s)
- Calculate the ΔCt for each of the mutation assays, having a Ct value 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
$\Delta Ct \leq 9$	Positive	If $\Delta Ct \leq 9$ and the mutation assay Ct is ≤ 39 , the sample is positive for the mutation
$\Delta Ct > 9$	Negative	If $\Delta Ct > 9$ 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 the “Instructions for use” for further troubleshooting.

- If no signal in neither PentaYellow nor PentaGreen is present, no amplification has taken place indicating low DNA amount or quality (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
- Too high Ct value in PentaGreen for the reference indicates that the amount of DNA is too low. If possible, repeat PCR with higher DNA input
- Fluorescence drift could result from either sample or instrument instabilities or air bubbles

The full version of the “Instructions for use” can be found at www.pentabase.com.

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