SENSISCREEN® READY-TO-USE CE IVD QUICK GUIDE

SensiScreen® assays for sensitive detection and identification of mutations in cancer

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1. Introduction

Please review the full protocol carefully before using the kit. For each sample, the mutation specific analyses are compared to a corresponding reference assay. The reference assay is used for calculating a delta Ct value to distinguish mutation-specific from non-specific amplification. Furthermore, an internal control is present in all the mixes to verify sample and PCR quality of mutation negative analyses.

2. Content

The kit comprises 1, 12 or 60 x 0.2 mL 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 minimum 6 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 per analysis 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

Cycles	Temperature	Time	Data channel (Ex./Em.)
1	95˚C	2 min	-
45	94˚C	15 sec	**PentaGreen™** (495 nm / 520 nm)
	60˚C	60 sec	**PentaYellow™** (533 nm / 557 nm)

7. Notes

- All test components should be stored as described in “Instruction for use” (storage section)
- Do not mix reagents from different lots
- Use one strip per patient per analysis. 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 vigorously to remove air bubbles
- Tube number one is marked with strip annotation and comprise a reference assay

DATA ANALYSIS

- Correct for "baseline drift" before setting the threshold. Please refer to the specific instrument guides for details
- Samples giving no signal for neither the assay (PentaGreen™) nor the internal control (PentaYellow™) are invalid. Setup a new real-time PCR for these
- Set threshold for PentaGreen™ at 10% of the signal strength at cycle 45 for the reference sample
- Verify reference Ct according to Table 2. **Please notice that Strip K1 (KRAS Exon2+3+4) and Strip N2 (NRAS Exon 2+3+4) contains two different references. Tube 1 contains reference for tube 2-3 and tube 4 contains reference for tube 5-8**

Table 2: Reference Ct validation

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 ≤34	Optimal	The amount of input DNA is optimal for the results of the mutations analysis
34< 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

- Analyse for mutations by looking at the Ct values for the mutation-specific reactions. **Disregard any signals with Ct >39**
- Calculate ΔCt between reference signal and the mutation-specific signals as the Ct value of the mutation-specific signal subtracted the Ct value of the reference signal (both PentaGreen™ labelled)

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

- Use the calculated ΔCt value to evaluate KRAS, NRAS and BRAF mutation status according to Table 3

Table 3: KRAS, NRAS, BRAF and EGFR analysis

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

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 or contact our scientists at support@pentabase.com for assistance.

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