

## INSTRUCTIONS FOR USE SENSISCREEN<sup>®</sup> FFPE ASSAYS

SensiScreen<sup>®</sup> assays for sensitive detection and identification of mutations in cancer



Version: 3.3

Date of Revision: February 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>
1.1 <i>Indications for use</i> .....	3
<b>2. SUMMARY AND EXPLANATION OF THE ASSAYS</b> .....	<b>3</b>
<b>3. TECHNOLOGY AND REAGENTS</b> .....	<b>6</b>
3.1 <i>HydrolyEasy™ Probes</i> .....	6
3.2 <i>SuPrimers™</i> .....	6
3.3 <i>BaseBlockers™</i> .....	6
<b>4. ASSAY FORMAT AND DESIGN</b> .....	<b>6</b>
4.1 <i>Format</i> .....	6
4.2 <i>Internal control assay</i> .....	12
4.3 <i>Reference assay</i> .....	12
4.4 <i>Mutation assay</i> .....	12
4.5 <i>Equipment and reagents not supplied with SensiScreen®</i> .....	12
<b>5. SAFETY, SHIPMENT AND STORAGE</b> .....	<b>13</b>
5.1 <i>Precautions</i> .....	13
5.2 <i>Shipment</i> .....	13
5.3 <i>Storage</i> .....	14
<b>6. SPECIMENS</b> .....	<b>14</b>
6.1 <i>Recommended procedure for extraction of gDNA from FFPE section</i> .....	14
<b>7. SENSISCREEN® PROTOCOL</b> .....	<b>14</b>
7.1 <i>Ready-to-use</i> .....	14
7.2 <i>Dispense Ready</i> .....	14
<b>8. DATA ANALYSIS</b> .....	<b>15</b>
8.1 <i>Adjusting the baseline</i> .....	15
8.2 <i>Determining the mutational status</i> .....	16
8.3 <i>Analysis protocol</i> .....	16
8.4 <i>Internal control analysis</i> .....	17
<b>9. TROUBLESHOOTING</b> .....	<b>17</b>
<b>10. REFERENCES</b> .....	<b>18</b>
<b>11. MANUFACTURER AND DISTRIBUTORS</b> .....	<b>18</b>
11.1 <i>Manufacturer</i> .....	18

11.2	<i>Technical assistance</i> .....	19
12.	<b>SENSISCREEN® PRODUCT OVERVIEW</b> .....	<b>19</b>
13.	<b>ADDITIONAL INFORMATION</b> .....	<b>20</b>
13.1	<i>Date of revision</i> .....	20
14.	<b>NOTES</b> .....	<b>21</b>

## Δ IMPORTANT:

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

### 1. INTENDED USE

SensiScreen® assays are intended for in vitro diagnosis of specific somatic mutations including single point mutations, insertions, deletions and translocations. These tests will provide an assessment of the presence of the examined mutations constituting down to 0.25% of a human genomic DNA (gDNA) sample (from formalin fixed paraffin-embedded tumor biopsies).

SensiScreen® assays are to be used by trained laboratory personnel in a professional laboratory environment with human gDNA samples (e.g. gDNA extracted from formalin fixed paraffin-embedded tissues from cancer). SensiScreen® assays **are not intended for diagnosing of cancer** but only as an aid to assist the oncologist’s treatment planning.

The tests are provided in one or more boxes containing all necessary components for use including an “Instructions for Use” and a “Quick guide”. The “Instructions for Use” is also available for download on our website: [www.pentabase.com](http://www.pentabase.com).

#### 1.1 INDICATIONS FOR USE

The obtained results of SensiScreen® assays are intended to assist in identifying the presence of certain somatic mutations in the Murine Sarcoma Viral (V-raf) Oncogene Homolog B1 (BRAF); Epidermal growth factor receptor (EGFR); proto-oncogene tyrosine kinase (KIT); Kirsten Rat Sarcoma Viral Oncogene Homolog (KRAS); Neuroblastoma Ras Viral Oncogene Homolog (NRAS) and Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha (PIK3CA) genes. These mutations occur with different frequencies in different cancers such as colorectal- (CRC), lung cancer (NSCLC) or malignant melanoma (MM). According to the literature, these mutations either affect the response to certain treatments or the prognosis [1-5]. Importantly, SensiScreen® is used for selecting a suitable treatment based on the patient’s mutational status and not intended for diagnosing of cancer. Furthermore, the mutational status should always be considered alongside other disease factors when making treatment decisions.

### 2. SUMMARY AND EXPLANATION OF THE ASSAYS

CE-IVD marked SensiScreen® assays are in accordance with EU Directive 98/79/EC on Medical Equipment for in vitro diagnostic. With SensiScreen®, it is possible to detect a variety of somatic mutations in the BRAF, EGFR, KIT, KRAS, NRAS and PIK3CA genes in a background of wild type genomic DNA, using real-time PCR analysis. SensiScreen® is based on PentaBase’s highly sensitive DNA technology and, provided use of a sufficient quality of DNA input (with sufficient copies of DNA), it is possible to detect down to approximately 0.25% of mutated gDNA in a background of wild type gDNA. Refer to Table 1 (below) for mutations detected by SensiScreen®.

BRAF mutations detected with SensiScreen®			
Assay	CDS mutation	Amino acid substitution	Cosmic ID
BRAF exon 15	c.1799_1800TG>AT	p.Val600Asp (V600D)	COSM477
	c.1799T>A	p.Val600Glu (V600E)	COSM476
	c.1798_1799GT>AA	p.Val600Lys (V600K)	COSM473
	c.1798_1799GT>AG	p.Val600Arg (V600R)	COSM474

EGFR mutations detected with SensiScreen®				
Assay	CDS mutation	Amino acid substitution	Cosmic ID	Assay
EGFR exon 18	c.2156G>C	p.Gly719Ala	COSM6239	
	c.2155G>A	p.Gly719Ser	COSM6252	
	c.2155G>T	p.Gly719Cys	COSM6253	
EGFR exon 19	c.2240_2251del12	p.L747_T751>S	COSM6210	
	c.2239_2247del9	p.L747_E749delLRE	COSM6218	
	c.2238_2255del18	p.E746_S752>D	COSM6220	
	c.2235_2249del15	p.E746_A750delELREA	COSM6223	
	c.2236_2250del15	p.E746_A750delELREA	COSM6225	
	c.2235_2246del12	p.E746_E749delELRE	COSM28517	
	c.2239_2256del18	p.L747_S752delLREATS	COSM6255	
	c.2237_2254del18	p.E746_S752>A	COSM12367	
	c.2240_2254del15	p.L747_T751delLREAT	COSM12369	
	c.2240_2257del18	p.L747_P753>S	COSM12370	
	c.2239_2248>C (complex)	p.L747_A750>P	COSM12382	
	c.2239_2251>C (complex)	p.L747_T751>P	COSM12383	
	c.2237_2255>T (complex)	p.E746_S752>V	COSM12384	
	c.2235_2255>AAT (complex)	p.E746_S752>I	COSM12385	
	c.2237_2252>T (complex)	p.E746_T751>V	COSM12386	
	c.2239_2258>CA (complex)	p.L747_P753>Q	COSM12387	
	c.2239_2256>CAA (complex)	p.L747_S752>Q	COSM12403	
	c.2237_2253>TTGCT (complex)	p.E746_T751>VA	COSM12416	
	c.2238_2252>GCA (complex)	p.L747_T751>Q	COSM12419	
	c.2238_2248>GC (complex)	p.L747_A750>P	COSM12422	
	c.2237_2251del15	p.E746_T751>A	COSM12678	
	c.2236_2253del18	p.E746_T751delELREAT	COSM12728	
	c.2235_2248>AATTC (complex)	p.E746_A750>IP	COSM13550	
	c.2235_2252>AAT (complex)	p.E746_T751>I	COSM13551	
	c.2235_2251>AATTC (complex)	p.E746_T751>IP	COSM13552	
	c.2237_2257>TCT (complex)	p.E746_P753>VS	COSM18427	
	c.2237_2251del15	p.L747_T751delLREAT	COSM23571	
	c.2233_2247del15	p.K745_E749delKELRE	COSM26038	
	c.2234_2248del15	p.K745_A750>T	COSM1190791	
	c.2236_2248>CAAC (complex)	p.E746_A750>QP	COSM13557	
	c.2232_2249del18	p.K745_A750delKELREA	COSM221565	
	c.2237_2253>TA (complex)	p.E746_T751>V	COSM133192	
	c.2239_2257>T (complex)	p.L747_P753>S	COSM133197	
c.2239_2253>AAT (complex)	p.L747_T751>N	COSM51503		
c.2236_2259>ATCTCG (complex)	p.E746_P753>IS	COSM133191		
EGFR exon 20	c.2369C>T	p.Thr790Met (T790M)	COSM6240	
	c.2303G>T	p.Ser768Ile	COSM6241	
	c.2300_2301insCAGCGTGGA	p.D770_N771insSVD	COSM3728433	Multiplex 1
	c.2302_2303insCGCTGGCCA	p.A767_S768insTLA	COSM12425	Multiplex 1
	c.2307_2308ins15	p.V769_D770insMASVD	COSM28638	Multiplex 1
	c.2307_2308insGCCAGCGTG	p.V769_D770insASV	COSM12376	Multiplex 1
	c.2308_2309insCCAGCGTGG	p.V769_D770insASV	COSM12426	Multiplex 1
	c.2308_2309insGGGTCGTGG	p.V769_D770insGVV	COSM18430	Multiplex 1
	c.2308_2309insGTT	p.D770>GY	COSM12427	Multiplex 1
	c.2309_2310AC>CCAGCGTGGAT	p.V769_D770insASV	COSM13558	Multiplex 1
	c.2310_2311insAGCGTGGAC	p.D770_N771insSVD	COSM85749	Multiplex 1
	c.2310_2311insGGACA	p.D770_N771insGT	COSM1238029	Multiplex 1
	c.2310_2311insGGTTT	p.D770_N771insGF	COSM655155	Multiplex 1
	c.2310_2311insGGT	p.D770_N771insG	COSM12378	Multiplex 1
	c.2310_2311insAACCCCCAC	p.H773_V774insNPH	COSM48920	Multiplex 1+2
	c.2310_2311ins9GCGTGGACA	p.D770_N771insSVD	COSM13428	Multiplex 2
	c.2316_2317insNNN	p.P772_H773insX	COSM21597	Multiplex 2
	c.2319_2320insAACCCCCAC	p.H773_V774insNPH	COSM12381	Multiplex 1+2
	c.2319_2320insCAC	p.H773_V774insH	COSM12377	Multiplex 2
	c.2319_2320insCCCCAC	p.H773_V774insPH	COSM12380	Multiplex 2
	c.2320_2321insCCACG	p.H773_V774insAH	COSM1238028	Multiplex 2
	c.2321_2322insCCACGT	p.V774_C775insHV	COSM18432	Multiplex 2

	c.2322_2323insCACGTG	p.V774_C775insHV	COSM22948	Multiplex 2
EGFR exon 21	c.2573T>G	p.Leu858Arg	COSM6224	
	c.2573_2574TG>GT	p.Leu858Arg	COSM12429	
	c.2582T>A	p.Leu861Gln	COSM6213	
<b>KIT mutations detected with SensiScreen®</b>				
<b>Assay</b>	<b>CDS mutation</b>	<b>Amino acid substitution</b>	<b>Cosmic ID</b>	
KIT D816V	c.2447A>T	Asp816Val	COSM1314	
<b>KRAS mutations detected with SensiScreen®</b>				
<b>Assay</b>	<b>CDS mutation</b>	<b>Amino acid substitution</b>	<b>Cosmic ID</b>	
KRAS exon 2	c.35G>C	p.Gly12Ala (G12A)	COSM522	
	c.35G>A	p.Gly12Asp (G12D)	COSM521	
	c.34G>C	p.Gly12Arg (G12R)	COSM518	
	c.34G>T	p.Gly12Cys (G12C)	COSM516	
	c.34G>A	p.Gly12Ser (G12S)	COSM517	
	c.35G>T	p.Gly12Val (G12V)	COSM520	
	c.38G>A	p.Gly13Asp (G13D)	COSM532	
	c.34_35GG>TT	p.Gly12Phe (G12F)	COSM512	
	c.34_35GG>AT	p.Gly12Ile (G12I)	COSM34144	
KRAS exon 3	c.176C>G	p.Ala59Gly (A59G)	COSM28518	
	c.175G>A	p.Ala59Thr (A59T)	COSM546	
	c.183A>C	p.Gln61His (Q61H1)	COSM554	
	c.183A>T	p.Gln61His (Q61H2)	COSM555	
	c.181C>G	p.Gln61Glu (Q61E)	COSM550	
	c.181C>A	p.Gln61Lys (Q61K)	COSM549	
	c.182A>T	p.Gln61Leu (Q61L)	COSM553	
	c.182A>G	p.Gln61Arg (Q61R)	COSM552	
KRAS exon 4	c.351A>C	p.Lys117Asn (K117N1)	COSM19940	
	c.351A>T	p.Lys117Asn (K117N2)	COSM28519	
	c.436G>C	p.Ala146Pro (A146P)	COSM19905	
	c.436G>A	p.Ala146Thr (A146T)	COSM19404	
	c.437C>T	p.Ala146Val (A146V)	COSM19900	
<b>NRAS mutations detected with SensiScreen®</b>				
<b>Assay</b>	<b>CDS mutation</b>	<b>Amino acid substitution</b>	<b>Cosmic ID</b>	
NRAS exon 2	c.35G>C	p.Gly12Ala (G12A)	COSM565	
	c.34G>T	p.Gly12Cys (G12C)	COSM562	
	c.35G>A	p.Gly12Asp (G12D)	COSM564	
	c.34G>C	p.Gly12Arg (G12R)	COSM561	
	c.34G>A	p.Gly12Ser (G12S)	COSM563	
	c.35G>T	p.Gly12Val (G12V)	COSM566	
	c.38G>C	p.Gly13Ala (G13A)	COSM575	
	c.37G>T	p.Gly13Cys (G13C)	COSM570	
	c.38G>A	p.Gly13Asp (G13D)	COSM573	
	c.37G>C	p.Gly13Arg (G13R)	COSM569	
	c.37G>A	p.Gly13Ser (G13S)	COSM571	
	c.38G>T	p.Gly13Val (G13V)	COSM574	
	NRAS exon 3	c.183A>T	p.Gln61His (Q61H1)	COSM585
c.183A>C		p.Gln61His (Q61H2)	COSM586	
c.181C>A		p.Gln61Lys (Q61K)	COSM580	
c.182A>T		p.Gln61Leu (Q61L)	COSM583	
c.182A>G		p.Gln61Arg (Q61R)	COSM584	
NRAS exon 4	c.351G>C	p.Lys117Asn (K117N1)	N/A	
	c.351G>T	p.Lys117Asn (K117N2)	N/A	
	c.436G>C	p.Ala146Pro (A146P)	(COSM4172577)	
	c.436G>A	p.Ala146Thr (A146T)	COSM27174	
	c.437C>T	p.Ala146Val (A146V)	COSM4170228	
<b>PIK3CA mutations detected with SensiScreen®</b>				
<b>Assay</b>	<b>CDS mutation</b>	<b>Amino acid substitution</b>	<b>Cosmic ID</b>	
PIK3CA	c.3140A>T	p.H1047L	COSM776	

	c.3140A>G	p.H1047R	COSM775
	c.3139C>T	p.H1047Y	COSM774

**Table 1.** List of mutations detected by SensiScreen® assays

### 3. TECHNOLOGY AND REAGENTS

SensiScreen® assays combine allele-specific PCR [6-7] 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. SensiScreen® assays contain both standard oligonucleotides and pentabase-modified oligonucleotides (HydrolEasy™ probes, SuPrimers™, and BaseBlockers™). Using SensiScreen®, somatic mutations can be detected quickly (in less than one and a half hour), sensitively (5-50 ng gDNA input per well/vial) and selectively (down to 0.25% mutation in wild type background of gDNA), by real-time PCR analysis.

#### 3.1 HYDROLEASY™ PROBES

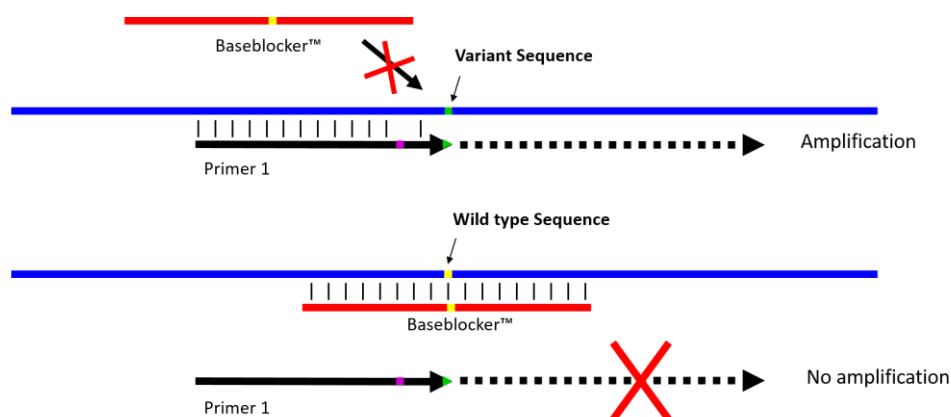
A **HydrolEasy™** probe is similar to a standard hydrolysis probe (also referred to as a TaqMan® probe) labeled with a fluorophore at the 5' end, a quencher at the 3' end, but with the addition of pentabases giving the probe a significantly improved signal-to-noise ratio, higher specificity and higher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

SensiScreen® FFPE assays are supplied in either "Ready-to-Use" or "Dispense Ready" versions and can be ordered as either Simplex or Multiplex configurations. SensiScreen® Ready-to-use assays are provided in either 1, 12 or 60 reactions in pre-aliquoted PCR strips (Table 2), while SensiScreen® Dispense Ready assays are provided in 20 or 50 reactions (Table 3).

**SensiScreen® assays contain the following reagents:**

*Reference assays*

- 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)
- Master mix (with no, low or high ROX™ included)

*Mutation assays*

- Mutation assay primer/probe/BaseBlocker™ mix (labeled with PentaGreen™, for detection on green channel)
- Internal control primer/probe mix (labeled with PentaYellow™, for detection in yellow channel)
- Master mix (with no, low or high ROX™ included)

<b>BRAF V600 Assays</b>					
Strip #	Gene	Tube #	Content	Mutations	Corresponding reference
B1	BRAF V600 Multiplex	A	<b>BRAF Reference 1</b>	V600E; V600D; V600K; V600R	BRAF Reference 1
		B	BRAF V600 Multiplex		
B2	BRAF V600 Simplex	A	<b>BRAF Reference 1</b>	V600D V600E V600K V600R	BRAF Reference 1 BRAF Reference 1 BRAF Reference 1 BRAF Reference 1
		B	BRAF V600 Simplex		
		C	BRAF V600 Simplex		
		D	BRAF V600 Simplex		
		E	BRAF V600 Simplex		
B3	BRAF V600E Simplex	A	<b>BRAF Reference 1</b>	V600E	BRAF Reference 1
		B	BRAF V600E Simplex		
<b>EGFR Assays</b>					
Strip #	Gene	Tube #	Content	Mutations	Corresponding reference
E1	EGFR exon 18+19+20+21	A	<b>EGFR Reference 1</b>	G719A; G719C; G719S 35 deletions. See table 1. S768I; L861Q T790M 13 insertions. See table 1. 9 insertions. See table 1. L858R	EGFR Reference 1 EGFR Reference 1 EGFR Reference 1 EGFR Reference 1 EGFR Reference 1 EGFR Reference 1 EGFR Reference 1
		B	EGFR G719 Multiplex		
		C	EGFR exon 19 Deletions		
		D	EGFR S768I + L861Q Multiplex		
		E	EGFR T790M Simplex		
		F	EGFR exon 20 Insertions 1		
		G	EGFR exon 20 Insertions 2		
		H	EGFR L858R Simplex		
E2	EGFR G719 Multiplex	A	<b>EGFR Reference 1</b>	G719A; G719C; G719S	EGFR Reference 1
		B	EGFR G719 Multiplex		
E3	EGFR G719 Simplex	A	<b>EGFR Reference 1</b>	G719A G719C G719S	EGFR Reference 1
		B	EGFR G719A Simplex		
		C	EGFR G719C Simplex		
		D	EGFR G719S Simplex		
E4	EGFR exon 19 Deletions	A	<b>EGFR Reference 2</b>	35 deletions. See table 1.	EGFR Reference 2
		B	EGFR exon 19 Deletions		
E5	EGFR S768I	A	<b>EGFR Reference 3</b>	S768I	EGFR Reference 3
		B	EGFR S768I Simplex		
E6	EGFR T790M	A	<b>EGFR Reference 4</b>	T790M	EGFR Reference 4
		B	EGFR T790M Simplex		
E7	EGFR exon 20 Insertions	A	<b>EGFR Reference 5</b>	13 insertions. See table 1. 9 insertions. See table 1.	EGFR Reference 5 EGFR Reference 5
		B	EGFR exon 20 Insertions 1		
		C	EGFR exon 20 Insertions 2		
E8	EGFR L858R	A	<b>EGFR Reference 6</b>	L858R	EGFR Reference 6
		B	EGFR L858R Simplex		
E9	EGFR L861Q	A	<b>EGFR Reference 7</b>	L861Q	EGFR Reference 7
		B	EGFR L861Q Simplex		
E10	EGFR exon 19 Deletions; T790M; L858R	A	<b>EGFR Reference 4</b>	35 deletions. See table1. T790M L858R	EGFR Reference 4 EGFR Reference 4 EGFR Reference 4
		B	EGFR exon 19 Deletions		
		C	EGFR T790M Simplex		
		D	EGFR L858R Simplex		



KIT D816V Assay					
Strip #	Gene	Tube #	Content	Mutations	Corresponding reference
I1*	KIT	A	KIT Reference 1	D816V	KIT Reference 1
		B	KIT Simplex 1		
KRAS Assays					
Strip #	Gene	Tube #	Content	Mutations	Corresponding reference
K1	KRAS exon 2+3+4 Multiplex	A	KRAS Reference 1	G12R; G12C; G12S; G12V G12A; G12D; G13D	KRAS Reference 1 KRAS Reference 1
		B	KRAS exon 2 Multiplex 1		
		C	KRAS exon 2 Multiplex 2		
		D	KRAS Reference 3		
		E	KRAS exon 3 Multiplex 1		
		F	KRAS exon 3 Multiplex 2		
		G	KRAS exon 4 Multiplex 1		
		H	KRAS exon 4 Multiplex 2		
K2	KRAS exon 2 Multiplex	A	KRAS Reference 1	G12R; G12C; G12S; G12V G12A; G12D; G13D	KRAS Reference 1 KRAS Reference 1
		B	KRAS exon 2 Multiplex 1		
		C	KRAS exon 2 Multiplex 2		
K3	KRAS exon 3 Multiplex	A	KRAS Reference 2	Q61H1; Q61K; Q61L; A59T Q61H2; Q61E; Q61R; A59G	KRAS Reference 2 KRAS Reference 2
		B	KRAS exon 3 Multiplex 1		
		C	KRAS exon 3 Multiplex 2		
K4	KRAS exon 4 Multiplex	A	KRAS Reference 3	K117N; K117N2 A146P; A146T; A146V	KRAS Reference 3 KRAS Reference 3
		B	KRAS exon 4 Multiplex 1		
		C	KRAS exon 4 Multiplex 2		
K5	KRAS exon 2 Simplex 1	A	KRAS Reference 1	G12R G12C G12S G12V	KRAS Reference 1 KRAS Reference 1 KRAS Reference 1 KRAS Reference 1
		B	KRAS exon 2 G12R Simplex		
		C	KRAS exon 2 G12C Simplex		
		D	KRAS exon 2 G12S Simplex		
		E	KRAS exon 2 G12V Simplex		
K6	KRAS exon 2 Simplex 2	A	KRAS Reference 1	G12A G12D G13D	KRAS Reference 1 KRAS Reference 1 KRAS Reference 1
		B	KRAS exon 2 G12A Simplex		
		C	KRAS exon 2 G12D Simplex		
		D	KRAS exon 2 G13D Simplex		
K7	KRAS exon 3 Simplex 1	A	KRAS Reference 2	Q61H1 Q61K Q61L A59T	KRAS Reference 2 KRAS Reference 2 KRAS Reference 2 KRAS Reference 2
		B	KRAS exon 3 Q61H1 Simplex		
		C	KRAS exon 3 Q61K Simplex		
		D	KRAS exon 3 Q61L Simplex		
		E	KRAS exon 3 A59T Simplex		
K8	KRAS exon 3 Simplex 2	A	KRAS Reference 2	Q61H2 Q61E Q61R A59G	KRAS Reference 2 KRAS Reference 2 KRAS Reference 2 KRAS Reference 2
		B	KRAS exon 3 Q61H2 Simplex		
		C	KRAS exon 3 Q61E Simplex		
		D	KRAS exon 3 Q61R Simplex		
		E	KRAS exon 3 A59G Simplex		
K9	KRAS exon 4 Simplex 1	A	KRAS Reference 3	K117N1 K117N2	KRAS Reference 3 KRAS Reference 3
		B	KRAS exon 4 K117N1 Simplex		
		C	KRAS exon 4 K117N2 Simplex		
K10	KRAS exon 4 Simplex 2	A	KRAS Reference 4	A146P A146T A146V	KRAS Reference 4 KRAS Reference 4 KRAS Reference 4
		B	KRAS exon 4 A146P Simplex		
		C	KRAS exon 4 A146T Simplex		
		D	KRAS exon 4 A146V Simplex		
NRAS Assays					
Strip #	Gene	Tube #	Content	Mutations	Corresponding reference
N1	NRAS exon 2+3+4 Multiplex	A	NRAS Reference 1	G12A; G12C; G12D; G12R; G12S; G12V G13A; G13C; G13D; G13R; G13S; G13V	NRAS Reference 1 NRAS Reference 1
		B	NRAS exon 2 Multiplex 1		
		C	NRAS exon 2 Multiplex 2		
		D	NRAS Reference 2		
		E	NRAS exon 3 Multiplex 1		
		F	NRAS exon 3 Multiplex 2		
		G	NRAS exon 4 Multiplex 1		
		H	NRAS exon 4 Multiplex 2		
N2	NRAS exon 2 Multiplex	A	NRAS Reference 1	G12A; G12C; G12D; G12R; G12S; G12V G13A; G13C; G13D; G13R; G13S; G13V	NRAS Reference 1 NRAS Reference 1
		B	NRAS exon 2 Multiplex 1		
		C	NRAS exon 2 Multiplex 2		
N3	NRAS exon 3 Multiplex	A	NRAS Reference 2	Q61H1; Q61H2; Q61K; Q61L; Q61R A59D; A59T	NRAS Reference 2 NRAS Reference 2
		B	NRAS exon 3 Multiplex 1		
		C	NRAS exon 3 Multiplex 2		
N4	NRAS exon 4 Multiplex	A	NRAS Reference 3	K117N1; K117N2 A146P; A146T; A146V	NRAS Reference 3 NRAS Reference 3
		B	NRAS exon 4 Multiplex 1		
		C	NRAS exon 4 Multiplex 2		
N5	NRAS exon 2 Simplex 1	A	Reference 1	G12A	NRAS Reference 1
		B	NRAS exon 2 G12A Simplex		

		<b>C</b>	NRAS exon 2 G12C Simplex	G12C	NRAS Reference 1
		<b>D</b>	NRAS exon 2 G12D Simplex	G12D	NRAS Reference 1
		<b>E</b>	NRAS exon 2 G12R Simplex	G12R	NRAS Reference 1
		<b>F</b>	NRAS exon 2 G12S Simplex	G12S	NRAS Reference 1
		<b>G</b>	NRAS exon 2 G12V Simplex	G12V	NRAS Reference 1
<b>N6</b>	<b>NRAS exon 2 Simplex 2</b>	<b>A</b>	<b>NRAS Reference 4</b>		NRAS Reference 4
		<b>B</b>	NRAS exon 2 G13A Simplex	G13A	NRAS Reference 4
		<b>C</b>	NRAS exon 2 G13C Simplex	G13C	NRAS Reference 4
		<b>D</b>	NRAS exon 2 G13D Simplex	G13D	NRAS Reference 4
		<b>E</b>	NRAS exon 2 G13R Simplex	G13R	NRAS Reference 4
		<b>F</b>	NRAS exon 2 G13S Simplex	G13S	NRAS Reference 4
		<b>G</b>	NRAS exon 2 G13V Simplex	G13V	NRAS Reference 4
<b>N7</b>	<b>NRAS exon 3 Simplex 1</b>	<b>A</b>	<b>NRAS Reference 2</b>		NRAS Reference 2
		<b>B</b>	NRAS exon 3 Q61H1 Simplex	Q61H1	NRAS Reference 2
		<b>C</b>	NRAS exon 3 Q61H2 Simplex	Q61H2	NRAS Reference 2
		<b>D</b>	NRAS exon 3 Q61K Simplex	Q61K	NRAS Reference 2
		<b>E</b>	NRAS exon 3 Q61L Simplex	Q61L	NRAS Reference 2
		<b>F</b>	NRAS exon 3 Q61R Simplex	Q61R	NRAS Reference 2
<b>N8</b>	<b>NRAS exon 3 Simplex 2</b>	<b>A</b>	<b>NRAS Reference 2</b>		NRAS Reference 2
		<b>B</b>	NRAS exon 3 A59D Simplex	A59D	NRAS Reference 2
		<b>C</b>	NRAS exon 3 A59T Simplex	A59T	NRAS Reference 2
<b>N9</b>	<b>NRAS exon 4 Simplex 1</b>	<b>A</b>	<b>NRAS Reference 3</b>		NRAS Reference 3
		<b>B</b>	NRAS exon 4 K117N1 Simplex	K117N1	NRAS Reference 3
		<b>C</b>	NRAS exon 4 K117N2 Simplex	K117N2	NRAS Reference 3
<b>N10</b>	<b>NRAS exon 4 Simplex 2</b>	<b>A</b>	<b>NRAS Reference 5</b>		NRAS Reference 5
		<b>B</b>	NRAS exon 4 A146P Simplex	A146P	NRAS Reference 5
		<b>C</b>	NRAS exon 4 A146T Simplex	A146T	NRAS Reference 5
		<b>D</b>	NRAS exon 4 A146V Simplex	A146V	NRAS Reference 5
<b>PIK3CA H1047 Assays</b>					
<b>Strip #</b>	<b>Gene</b>	<b>Tube #</b>	<b>Content</b>	<b>Mutations</b>	<b>Corresponding reference</b>
<b>P1*</b>	<b>PIK3CA Multiplex</b>	<b>A</b>	<b>PIK3CA Reference 1</b>		PIK3CA Reference 1
		<b>B</b>	PIK3CA Multiplex	H1047R; H1047Y, H1047L	PIK3CA Reference 1
<b>P2*</b>	<b>PIK3CA Simplex</b>	<b>A</b>	<b>PIK3CA Reference 1</b>		PIK3CA Reference 1
		<b>B</b>	PIK3CA H1047L Simplex	H1047L	PIK3CA Reference 1
		<b>C</b>	PIK3CA H1047R Simplex	H1047R	PIK3CA Reference 1
		<b>D</b>	PIK3CA H1047Y Simplex	H1047Y	PIK3CA Reference 1

**Table 2:** List of SensiScreen® FFPE Ready-to-use assays. Each tube contains 20 µL in total (7,5 µL primer/probe-mix and 12,5 µL master mix). \*Research use only.

Gene	Tube #	Content	Mutations	Corresponding reference	Volume 20x	Volume 50x
<b>BRAF V600 Multiplex</b>	1	<b>BRAF Reference 1</b>			150 µL	375 µL
	2	BRAF V600 Multiplex	V600E; V600D; V600K; V600R	BRAF Reference 1	150 µL	375 µL
	3	Mastermix			500 µL	1250 µL
<b>BRAF V600 Simplex</b>	1	<b>BRAF Reference 1</b>			150 µL	375 µL
	2	BRAF V600D Simplex	V600D	BRAF Reference 1	150 µL	375 µL
	3	BRAF V600E Simplex	V600E	BRAF Reference 1	150 µL	375 µL
	4	BRAF V600K Simplex	V600K	BRAF Reference 1	150 µL	375 µL
	5	BRAF V600R Simplex	V600R	BRAF Reference 1	150 µL	375 µL
	6-7	Mastermix			1250 µL	3125 µL
<b>BRAF V600E Simplex</b>	1	<b>BRAF Reference 1</b>			150 µL	375 µL
	2	BRAF V600E Simplex	V600E	BRAF Reference 1	150 µL	375 µL
	3	Mastermix			500 µL	1250 µL
<b>EGFR Assays</b>						
Gene	Tube #	Content	Mutations	Corresponding reference	Volume 20x	Volume 50x
<b>EGFR Exon 18+19+20+21</b>	1	<b>EGFR Reference 1</b>			150 µL	375 µL
	2	EGFR G719 Multiplex	G719A; G719C; G719S	EGFR Reference 1	150 µL	375 µL
	3	EGFR exon 19 Deletions	35 deletions. See table 1.	EGFR Reference 1	150 µL	375 µL
	4	EGFR S768I + L861Q Multiplex	S768I; L861Q	EGFR Reference 1	150 µL	375 µL
	5	EGFR T790M Simplex	T790M	EGFR Reference 1	150 µL	375 µL
	6	EGFR exon 20 Insertions 1	13 insertions. See table 1.	EGFR Reference 1	150 µL	375 µL
	7	EGFR exon 20 Insertions 2	9 insertions. See table 1.	EGFR Reference 1	150 µL	375 µL
	8	EGFR L858R Simplex	L858R	EGFR Reference 1	150 µL	375 µL
	9-11	Mastermix			2000 µL	5000 µL
<b>EGFR G719 Multiplex</b>	1	<b>EGFR Reference 1</b>			150 µL	375 µL
	2	EGFR G719 Multiplex	G719A; G719C; G719S	EGFR Reference 1	150 µL	375 µL
	3	Mastermix			500 µL	1250 µL

EGFR G719 Simplex	1	<b>EGFR Reference 1</b>	G719A G719C G719S	EGFR Reference 1 EGFR Reference 1 EGFR Reference 1	150 µL	375 µL
	2	EGFR exon 18 G719A Simplex			150 µL	375 µL
	3	EGFR exon 18 G719C Simplex			150 µL	375 µL
	4	EGFR exon 18 G719S Simplex			150 µL	375 µL
	5-6	Mastermix			1000 µL	2500 µL
EGFR Exon 19 Deletions	1	<b>EGFR Reference 2</b>	35 deletions. See table 1.	EGFR Reference 2	150 µL	375 µL
	2	EGFR exon 19 Multiplex			150 µL	375 µL
	3	Mastermix			500 µL	1250 µL
EGFR S768I	1	<b>EGFR Reference 3</b>	S768I	EGFR Reference 3	150 µL	375 µL
	2	EGFR S768I Simplex			150 µL	375 µL
	3	Mastermix			500 µL	1250 µL
EGFR T790M	1	<b>EGFR Reference 4</b>	T790M	EGFR Reference 4	150 µL	375 µL
	2	EGFR T790M Simplex			150 µL	375 µL
	3	Mastermix			500 µL	1250 µL
EGFR Exon 20 Insertions	1	<b>EGFR Reference 5</b>	13 insertions. See table 1. 9 insertions. See table 1.	EGFR Reference 5 EGFR Reference 5	150 µL	375 µL
	2	EGFR exon 20 Multiplex 1			150 µL	375 µL
	3	EGFR exon 20 Multiplex 2			150 µL	375 µL
	4	Mastermix			750 µL	1875 µL
EGFR L858R	1	<b>EGFR Reference 6</b>	L858R	EGFR Reference 6	150 µL	375 µL
	2	EGFR L858R Simplex			150 µL	375 µL
	3	Mastermix			500 µL	1250 µL
EGFR L861Q	1	<b>EGFR Reference 7</b>	L861Q	EGFR Reference 7	150 µL	375 µL
	2	EGFR L861Q Simplex			150 µL	375 µL
	3	Mastermix			500 µL	1250 µL
EGFR exon 19 deletions; T790M; L858R	1	<b>EGFR Reference 4</b>	35 deletions. See table below. T790M L858R	EGFR Reference 4 EGFR Reference 4 EGFR Reference 4	150 µL	375 µL
	2	EGFR exon 19 Deletions			150 µL	375 µL
	3	EGFR T790M Simplex			150 µL	375 µL
	4	EGFR L858R Simplex			150 µL	375 µL
	5-6	Mastermix			1000 µL	2500 µL
<b>KIT D816V Assay</b>						
<b>Gene</b>	<b>Tube #</b>	<b>Content</b>	<b>Mutations</b>	<b>Corresponding reference</b>	<b>Volume 20x</b>	<b>Volume 50x</b>
KIT D816V*	1	<b>KIT Reference 1</b>	D816V	KIT Reference 1	150 µL	375 µL
	2	KIT Simplex 1			150 µL	375 µL
	3	Mastermix			500 µL	1250 µL
<b>KRAS Assays</b>						
<b>Gene</b>	<b>Tube #</b>	<b>Content</b>	<b>Mutations</b>	<b>Corresponding reference</b>	<b>Volume 20x</b>	<b>Volume 50x</b>
KRAS exon 2+3+4 Multiplex	1	<b>KRAS Reference 1</b>	G12R; G12C; G12S; G12V G12A; G12D; G13D  Q61H1; Q61K; Q61L; A59T Q61H2; Q61E; Q61R; A59G K117N; K117N2 A146P; A146T; A146V	KRAS Reference 1 KRAS Reference 1  KRAS Reference 3 KRAS Reference 3 KRAS Reference 3 KRAS Reference 3	150 µL	375 µL
	2	KRAS exon 2 Multiplex 1			150 µL	375 µL
	3	KRAS exon 2 Multiplex 2			150 µL	375 µL
	4	<b>KRAS Reference 3</b>			160 µL	375 µL
	5	KRAS exon 3 Multiplex 1			150 µL	375 µL
	6	KRAS exon 3 Multiplex 2			150 µL	375 µL
	7	KRAS exon 4 Multiplex 1			150 µL	375 µL
	8	KRAS exon 4 Multiplex 2			150 µL	375 µL
	9-11	Mastermix			2000 µL	5000 µL
KRAS exon 2 Multiplex	1	<b>KRAS Reference 1</b>	G12R; G12C; G12S; G12V G12A; G12D; G13 D	KRAS Reference 1 KRAS Reference 1	150 µL	375 µL
	2	KRAS exon 2 Multiplex 1			150 µL	375 µL
	3	KRAS exon 2 Multiplex 2			150 µL	375 µL
	4	Mastermix			750 µL	1875 µL
KRAS exon 3 Multiplex	1	<b>KRAS Reference 2</b>	Q61H1; Q61K; Q61L; A59T Q61H2; Q61E; Q61R; A59G	KRAS Reference 2 KRAS Reference 2	150 µL	375 µL
	2	KRAS exon 3 Multiplex 1			150 µL	375 µL
	3	KRAS exon 3 Multiplex 2			150 µL	375 µL
	4	Mastermix			750 µL	1875 µL
KRAS exon 4 Multiplex	1	<b>KRAS Reference 3</b>	K117N; K117N2 A146P; A146T; A146V	KRAS Reference 2 KRAS Reference 2	150 µL	375 µL
	2	KRAS exon 4 Multiplex 1			150 µL	375 µL
	3	KRAS exon 4 Multiplex 2			150 µL	375 µL
	4	Mastermix			750 µL	1875 µL
KRAS exon 2 Simplex 1	1	<b>KRAS Reference 1</b>	G12R G12C G12S G12V	KRAS Reference 1 KRAS Reference 1 KRAS Reference 1 KRAS Reference 1	150 µL	375 µL
	2	KRAS exon 2 G12R Simplex			150 µL	375 µL
	3	KRAS exon 2 G12C Simplex			150 µL	375 µL
	4	KRAS exon 2 G12S Simplex			150 µL	375 µL
	5	KRAS exon 2 G12V Simplex			150 µL	375 µL
	6-7	Mastermix			1250 µL	3125 µL
KRAS exon 2 Simplex 2	1	<b>KRAS Reference 1</b>	G12A G12D G13D	KRAS Reference 1 KRAS Reference 1 KRAS Reference 1	150 µL	375 µL
	2	KRAS exon 2 G12A Simplex			150 µL	375 µL
	3	KRAS exon 2 G12D Simplex			150 µL	375 µL
	4	KRAS exon 2 G13D Simplex			150 µL	375 µL
	5-6	Mastermix			1000 µL	2500 µL
	3	KRAS exon 3 Q61H1 Simplex			150 µL	375 µL
KRAS exon 3 Simplex 1	1	<b>KRAS Reference 2</b>	Q61H1 Q61K	KRAS Reference 2 KRAS Reference 2	150 µL	375 µL
	2	KRAS exon 3 Q61H1 Simplex			150 µL	375 µL
	3	KRAS exon 3 Q61K Simplex			150 µL	375 µL

	4 5 6-7	KRAS exon 3 Q61L Simplex KRAS exon 3 A59T Simplex Mastermix	Q61L A59T	KRAS Reference 2 KRAS Reference 2	150 µL 150 µL 1250 µL	375 µL 375 µL 3125 µL
<b>KRAS exon 3 Simplex 2</b>	1 2 3 4 5 6-7	<b>KRAS Reference 2</b> KRAS exon 3 Q61H2 Simplex KRAS exon 3 Q61E Simplex KRAS exon 3 Q61R Simplex KRAS exon 3 A59G Simplex Mastermix	Q61H2 Q61E Q61R A59G	KRAS Reference 2 KRAS Reference 2 KRAS Reference 2 KRAS Reference 2	150 µL 150 µL 150 µL 150 µL 1250 µL	375 µL 375 µL 375 µL 375 µL 3125 µL
<b>KRAS exon 4 Simplex 1</b>	1 2 3 4	<b>KRAS Reference 3</b> KRAS exon 4 K117N1 Simplex KRAS exon 4 K117N2 Simplex Mastermix	K117N K117N2	KRAS Reference 3 KRAS Reference 3	150 µL 150 µL 150 µL 750 µL	375 µL 375 µL 375 µL 1875 µL
<b>KRAS exon 4 Simplex 2</b>	1 2 3 4 5-6	<b>KRAS Reference 4</b> KRAS exon 4 A146P Simplex KRAS exon 4 A146T Simplex KRAS exon 4 A146V Simplex Mastermix	A146P A146T A146V	KRAS Reference 4 KRAS Reference 4 KRAS Reference 4	150 µL 150 µL 150 µL 1000 µL	375 µL 375 µL 375 µL 2500 µL
<b>NRAS Assays</b>						
<b>Gene</b>	<b>Tube #</b>	<b>Content</b>	<b>Mutations</b>	<b>Corresponding reference</b>	<b>Volume 20x</b>	<b>Volume 50x</b>
<b>NRAS exon 2+3+4 Multiplex</b>	1 2 3 4 5 6 7 8 9-11	<b>NRAS Reference 1</b> NRAS exon 2 Multiplex 1 NRAS exon 2 Multiplex 2 <b>NRAS Reference 2</b> NRAS exon 3 Multiplex 1 NRAS exon 3 Multiplex 2 NRAS exon 4 Multiplex 1 NRAS exon 4 Multiplex 1 Mastermix	G12A; G12C; G12D; G12R; G12S; G12V G13A; G13C; G13D; G13R; G13S; G13V  Q61H1; Q61H2; Q61K; Q61L; Q61R A59D; A59T K117N1; K117N2 A146P; A146T; A146V	NRAS Reference 1 NRAS Reference 1  NRAS Reference 2 NRAS Reference 2 NRAS Reference 2 NRAS Reference 2	150 µL 150 µL 150 µL 150 µL 150 µL 150 µL 150 µL 2000 µL	375 µL 375 µL 375 µL 375 µL 375 µL 375 µL 375 µL 5000 µL
<b>NRAS exon 2 Multiplex</b>	1 2 3 4	<b>NRAS Reference 1</b> NRAS exon 2 Multiplex 1 NRAS exon 2 Multiplex 2 Mastermix	G12A; G12C; G12D; G12R; G12S; G12V G13A; G13C; G13D; G13R; G13S; G13V	NRAS Reference 1 NRAS Reference 1	150 µL 150 µL 150 µL 750 µL	375 µL 375 µL 375 µL 1875 µL
<b>NRAS exon 3 Multiplex</b>	1 2 3 4	<b>NRAS Reference 2</b> NRAS exon 3 Multiplex 1 NRAS exon 3 Multiplex 2 Mastermix	Q61H1; Q61H2; Q61K; Q61L; Q61R A59D; A59T	NRAS Reference 2 NRAS Reference 2	150 µL 150 µL 150 µL 750 µL	375 µL 375 µL 375 µL 1875 µL
<b>NRAS exon 4 Multiplex</b>	1 2 3 4	<b>NRAS Reference 3</b> NRAS exon 4 Multiplex 1 NRAS exon 4 Multiplex 1 Mastermix	K117N1; K117N2 A146P; A146T; A146V	NRAS Reference 3 NRAS Reference 3	150 µL 150 µL 150 µL 700 µL	375 µL 375 µL 375 µL 1250 µL
<b>NRAS exon 2 Simplex 1</b>	1 2 3 4 5 6 7 8-10	<b>Reference 1</b> NRAS exon 2 G12A Simplex NRAS exon 2 G12C Simplex NRAS exon 2 G12D Simplex NRAS exon 2 G12R Simplex NRAS exon 2 G12S Simplex NRAS exon 2 G12V Simplex Mastermix	G12A G12C G12D G12R G12S G12V	NRAS Reference 1 NRAS Reference 1 NRAS Reference 1 NRAS Reference 1 NRAS Reference 1 NRAS Reference 1	150 µL 150 µL 150 µL 150 µL 150 µL 150 µL 1750 µL	375 µL 375 µL 375 µL 375 µL 375 µL 375 µL 4375 µL
<b>NRAS exon 2 Simplex 2</b>	1 2 3 4 5 6 7 8-10	<b>NRAS Reference 4</b> NRAS exon 2 G13A Simplex NRAS exon 2 G13C Simplex NRAS exon 2 G13D Simplex NRAS exon 2 G13R Simplex NRAS exon 2 G13S Simplex NRAS exon 2 G13V Simplex Mastermix	G13A G13C G13D G13R G13S G13V	NRAS Reference 4 NRAS Reference 4 NRAS Reference 4 NRAS Reference 4 NRAS Reference 4 NRAS Reference 4	150 µL 150 µL 150 µL 150 µL 150 µL 150 µL 1750 µL	375 µL 375 µL 375 µL 375 µL 375 µL 375 µL 4375 µL
<b>NRAS exon 3 Simplex 1</b>	1 2 3 4 5 6 7-8	<b>NRAS Reference 2</b> NRAS exon 3 Q61H1 Simplex NRAS exon 3 Q61H2 Simplex NRAS exon 3 Q61K Simplex NRAS exon 3 Q61L Simplex NRAS exon 3 Q61R Simplex Mastermix	Q61H1 Q61H2 Q61K Q61L Q61R	NRAS Reference 2 NRAS Reference 2 NRAS Reference 2 NRAS Reference 2 NRAS Reference 2	150 µL 150 µL 150 µL 150 µL 150 µL 1500 µL	375 µL 375 µL 375 µL 375 µL 375 µL 3750 µL
<b>NRAS exon 3 Simplex 2</b>	1 2 3 4	<b>NRAS Reference 2</b> NRAS exon 3 A59D Simplex NRAS exon 3 A59T Simplex Mastermix	A59D A59T	NRAS Reference 2 NRAS Reference 2	150 µL 150 µL 150 µL 750 µL	375 µL 375 µL 375 µL 1875 µL
<b>NRAS exon 4 Simplex 1</b>	1 2 3 4	<b>NRAS Reference 3</b> NRAS exon 4 K117N1 Simplex NRAS exon 4 K117N2 Simplex Mastermix	K117N1 K117N2	NRAS Reference 3 NRAS Reference 3	150 µL 150 µL 150 µL 750 µL	375 µL 375 µL 375 µL 1875 µL
<b>NRAS exon 4</b>	1	<b>NRAS Reference 5</b>			150 µL	375 µL

<b>Simplex 2</b>	2	NRAS exon 4 A146P Simplex	A146P	NRAS Reference 5	150 µL	375 µL
	3	NRAS exon 4 A146T Simplex	A146T	NRAS Reference 5	150 µL	375 µL
	4	NRAS exon 4 A146V Simplex	A146V	NRAS Reference 5	150 µL	375 µL
	5-6	Mastermix			1000 µL	2500 µL
<b>PIK3CA H1047 Assays</b>						
<b>Gene</b>	<b>Tube #</b>	<b>Content</b>	<b>Mutations</b>	<b>Corresponding reference</b>	<b>Volume 20x</b>	<b>Volume 50x</b>
<b>PIK3CA Multiplex*</b>	1	<b>PIK3CA Reference 1</b>			150 µL	375 µL
	2	PIK3CA Multiplex	H1047L, H1047R, H1047Y	PIK3CA Reference 1	150 µL	375 µL
	3	Mastermix			300 µL	1250 µL
<b>PIK3CA Simplex*</b>	1	<b>PIK3CA Reference 1</b>			150 µL	375 µL
	2	PIK3CA H1047L Simplex	H1047L	PIK3CA Reference 1	150 µL	375 µL
	3	PIK3CA H1047R Simplex	H1047R	PIK3CA Reference 1	150 µL	375 µL
	4	PIK3CA H1047Y Simplex	H1047Y	PIK3CA Reference 1	150 µL	375 µL
	5-6	Mastermix			1000 µL	2500 µL

**Table 3.** List of SensiScreen FFPE Dispense-Ready assays. Each tube contains reagents for either 20 or 50 reactions. \*Research use only.

#### 4.2 INTERNAL CONTROL ASSAY

An internal control assay is included in all the primer-probe mixes of the different assays and comprise a HydrolEasy™ probe labeled with PentaYellow™ (measured on the same fluorescence channel as HEX™, VIC® and 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 gDNA in the sample. The reference assay contains a HydrolEasy™ probe labeled with PentaGreen™ (measured on the same channel as FAM™), 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 assays of interest. See section 8 “Data Analysis” for more details.

#### 4.4 MUTATION ASSAY

The mutation assay(s) (see Table 2 and 3) targets the genomic region containing the mutation(s) of interest and is used to determine the presence of the mutation(s) in a sample. Mutation assays all contain a HydrolEasy™ probe labelled with PentaGreen™ (measured at the FAM™ channel), BaseBlockers™ (to reduce or eliminate non-specific amplification of wild type), a mutation-specific primer set, and an internal control assay. The mutation-assays are optimized to the conditions specified in section 7 and it is therefore important that these are followed to avoid misleading results. The difference in the Ct value of the reference and the Ct value of the mutation-specific assay(s) is 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 SENSI SCREEN®

The use of SensiScreen® 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)

\*SensiScreen® has been validated on the following real-time PCR instruments: MyGo Pro and MyGo Mini (IT-IS Life Science Ltd.); Rotorgene (Qiagen); CFX96 (BioRad); Mx3000P and Mx3005P (Stratagene); PikoReal (Thermo Fisher); ABI 7500 and PRISM®7900HT (Applied Biosystems), Mic (bio molecular systems) and Lightcycler®96 and 480 (Roche). We recommend that one of these systems are used, but other instruments are likely applicable. ^ SensiScreen® ready-to-use assays are pre-dispensed in PCR strips (can be provided in either 0.1 mL or 0.2 mL strip tubes).

## 5. SAFETY, SHIPMENT AND STORAGE

---

General laboratory precautions should be taken. SensiScreen® 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 SensiScreen® assays:

- The assays are only for *in vitro* diagnostic
- SensiScreen® assays are not intended for diagnosing any type of cancer, but only as a supplement for other prognostic factors for the selection of patients who might benefit from a specific treatment (companion diagnostics)
- The mutational status determined by SensiScreen® assays 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 SensiScreen® 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 SensiScreen® performance. For more information, see section 4.5 “Equipment and Reagents not supplied with SensiScreen®”
- 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

SensiScreen® Ready-to-use assays are shipped on dry ice while Dispense Ready assays are shipped on either dry ice or blue ice. If the SensiScreen® 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

SensiScreen® 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 fresh, frozen or formalin fixed paraffin-embedded (FFPE) tumor sections or similar. The samples should be collected and stored after standard pathology methodologies to ensure optimal quality. Extracted gDNA should be stored at maximum -15°C until use.

### 6.1 RECOMMENDED PROCEDURE FOR EXTRACTION OF GDNA FROM FFPE SECTION

Several methods to extract gDNA from FFPE material can be used. Different methods have been validated under the development and validation of SensiScreen®, among other the Maxwell® 16 FFPE Plus LEV DNA Purification Kit (Promega, Cat. #AS2000 and AS3000). Regardless of method, it is recommended to follow the manufacturers protocol for gDNA extraction.

## 7. SENSISCREEN® PROTOCOL

---

Before using the assay, it is recommended to carefully read the full “Instructions for use”. When using SensiScreen® 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 (See Table 2 and 3). These should be analyzed in the same PCR run to ensure minimal variation.

### 7.1 READY-TO-USE

- Thaw the reaction mixtures and spin down
- Add 5 µL extracted gDNA (1-10 ng/µL) to the mutation assay(s) and the corresponding reference in this order. Mix by pipetting. It is recommended to include a no template control (NTC) in each run. Add sterile water instead of gDNA
- Close lids and spin down
- Place the strips into the instrument and run the protocol described in Table 5
- Analyze the samples in accordance with the analysis rules. For more information, see section 8 “Data analysis”

### 7.2 DISPENSE READY

- Thaw the reaction mixtures, mix and spin down
- Add 12.5 µL master mix to all tubes/wells
- Add 7.5 µL of reference mix or mutant mix to the tube/well and mix carefully by pipetting (see Table 4 for layout example)



- Add 5  $\mu\text{L}$  extracted gDNA (1-10  $\text{ng}/\mu\text{L}$ ) from each sample to the mutation assays and the corresponding reference. Mix by pipetting. It is recommended to include a NTC in each run. Add sterile water to the NTC instead of gDNA
- Seal all tubes/wells and spin down. Make sure that there are no bobbles in the solutions
- Place all the tubes/plate in the instrument and run the protocol as described in Table 5
- Analyze the samples in accordance with the analysis guidelines. For more information, see section 8 “Data analysis”

Example of a 96 well layout for KRAS exon 2 Dispense Ready Assay												
	1	2	3	4	5	6	7	8	9	10	11	12
Reference	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Sample 11	NTC
G12R	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Sample 11	NTC
G12C	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Sample 11	NTC
G12V	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Sample 11	NTC
G12A	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Sample 11	NTC
G12D	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Sample 11	NTC
G13D	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Sample 11	NTC

Table 4. SensiScreen® Dispense Ready setup example.

Protocol	Temperature	Time	Number of 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) Measure fluorescence intensity at the end of each cycle
	60°C	60 sec		

Table 5. SensiScreen® Real-time PCR protocol.

## 8. DATA ANALYSIS

In SensiScreen® 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). The Ct value is reflecting the DNA amount and any PCR inhibitors present in a sample.

### 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 6.

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 6. Setting the threshold.



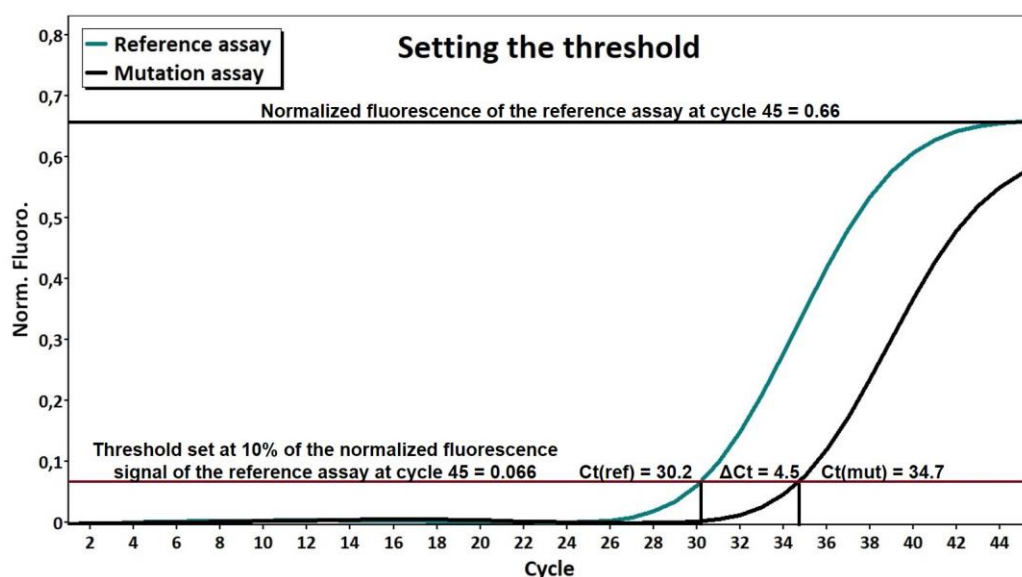
## 8.2 DETERMINING THE MUTATIONAL STATUS

To determine if the sample is wild type or mutated, a  $\Delta Ct$  value is calculated for each mutation-specific assay and is defined as the difference between the Ct value of the mutation assay subtracted the Ct value of the corresponding reference assay (Figure 2).

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

A sample is positive for a given mutation if the Ct of the reference assay is between 25 and 36, the Ct of the mutation assay is equal to or lower than 39, and the  $\Delta Ct$  value is equal to or lower than 9 (Table 8).

**Note:** In rare cases, a sample can contain multiple mutations. In these cases, more than one mutation-specific assay will be positive using the above formula. The samples will be positive when the respective  $\Delta Ct$  values are within the specified range for each mutation.



**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(s). In the shown example,  $\Delta Ct = Ct(\text{mut}) - Ct(\text{ref}) = 34.7 - 30.2 = 4.5$ . Thus, the sample is positive for the mutation analyzed.

## 8.3 ANALYSIS PROTOCOL

Use the following protocol to determine the mutational status:

1. Analyse 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 fluorescence signal at cycle 45 (Figure 2). Add any significant assay baseline fluorescence at cycle 20 to the threshold value (Table 6)
4. If NTC samples have been included in the run, verify that no signal is seen before Ct = 38 for the reference and Ct = 40 for mutation assay(s). 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 mutation assay(s) and the corresponding reference assay
6. Validate that the reference sample is suitable for analysis cf. Table 7

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 mutation 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

**Table 7:** Acceptable Ct values for the reference assay.

- Calculate  $\Delta Ct$  for each of the mutations having a Ct value equal to or lower than 39. A sample is positive for the mutation(s) of interest if the  $\Delta Ct$  is equal to or below 9 and negative if the  $\Delta Ct$  is above 9 cf. Table 8.

$\Delta Ct$ for assay	Conclusion	Comments
$\Delta Ct \leq 9$	Positive	The sample is mutation positive if $\Delta Ct \leq 9$ and Ct $\leq 39$
$\Delta Ct > 9$	Negative	The sample is mutation negative if $\Delta Ct \geq 9$ or Ct $\leq 39$

**Table 8:** Mutation analysis

For mutation positive samples, the theoretical mutation frequency based on the calculated  $\Delta Ct$  value is shown in table 9.

$\Delta Ct$	Mutation frequency
$\Delta Ct 3$	$\approx 10\%$
$\Delta Ct 7$	$\approx 1\%$
$\Delta Ct 8$	$\approx 0.5\%$
$\Delta Ct 8.5$	$\approx 0.3\%$
$\Delta Ct 9$	$\approx 0.2\%$

**Table 9:** Theoretical conversion of  $\Delta Ct$

#### 8.4 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 10 below covers some of the most frequent questions and problems that can occur with the use of SensiScreen® 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 or your local distributor. For contact details, see section 11" Manufacturer and Distributors"
No internal control signal (PentaYellow™)	There is no lower threshold for internal control assay. 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 poor quality of DNA or the presence of PCR inhibitors. Repeat the PCR with higher DNA quality and quantity. If there is a signal in the reference assay (in the green channel) with Ct<29 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 DNA has been used. If there is no signal (before Ct = 39/(40)) in the mutation-specific assays either, the purification of DNA 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 DNA.
No signal fom mutation-specific assays (PentaGreen™)	Check that there is signal from the internal control assay (yellow channel). If there is a signal, this sample does not comprise the specific mutation
$Ct_{reference} < 25$	The amount of input gDNA is too high. This can affect the performance of SensiScreen® Repeat the PCR with lower input of gDNA if possible
$31 < Ct_{reference} < 36$	The amount of input gDNA is lower than the recommended. If possible, repeat PCR with higher input of gDNA. If the mutation-specific assay is positive, the sample is most likely mutated
$Ct_{reference} > 36$	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

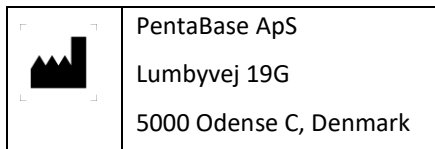
**Table 10:** Troubleshooting

## 10. REFERENCES

- van Krieken et al.** KRAS mutation testing for predicting response to anti-EGFR therapy for colorectal carcinoma: proposal for a European quality assurance program. *Virchows Arch.* (2008) 453:417-431
- Custodio, Feliu.** Prognostic and predictive biomarkers for epidermal growth factor receptor-targeted therapy in colorectal cancer: beyond KRAS mutations. *Crit. Rev. Oncol. Hematol.* (2013) 85:45-81.
- Rekhtman et al.** Molecular Testing for Selection of Patients With Lung Cancer for Epidermal Growth Factor Receptor and Anaplastic Lymphoma Kinase Tyrosine Kinase Inhibitors: American Society of Clinical Oncology Endorsement of the College of American Pathologists/International Society for the Study of Lung Cancer/Association of Molecular Pathologists Guideline. *Journal of Clinical Oncology* October 13, 2014.
- Fisher, Larkin.** Vemurafenib: a new treatment for BRAF-V600 mutated advanced melanoma. *Dove Press journal: Cancer Management and Research*, August 7, 2012.
- Van Cutsem et al.** ESMO consensus guidelines for the management of patients with metastatic colorectal cancer. *Annals of Oncology* 0:1-38, 2016
- Lang et al.** Optimized Allele-Specific Real-Time PCR Assays for the Detection of Common Mutations in KRAS and BRAF. *J. Mol. Diagn.* (2011) Jan 23;13(1):23-28.
- Riva et al.** SensiScreen® KRAS exon 2-sensitive simplex and multiplex real-time PCR-based assays for detection of KRAS exon 2 mutations. *PLOS one*, June 21, 2017.

## 11. MANUFACTURER AND DISTRIBUTORS

### 11.1 MANUFACTURER



## 11.2 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).

## 12. SENSISCREEN® PRODUCT OVERVIEW

Gene	SensiScreen® FFPE Ready-to-use	Strip #	Catalogue # 12; 60 reactions	SensiScreen® FFPE Dispense Ready	Catalogue # 20; 50 reactions
<b>BRAF</b>	V600 Multiplex Ready-to-use	B1	1831-1832	V600 Multiplex Dispense Ready	1398-1399
	V600 Simplex Ready-to-use (V600E, V600D, V600R and V600K)	B2	1836-1837	V600 Simplex Dispense Ready (V600E, V600D, V600R and V600K)	1402-1403
	V600E Simplex Ready-to-use	B3	1841-1842	V600E Simplex Dispense Ready	1400-1401
<b>EGFR</b>	Exon 18+19+20+21 Multiplex Ready to use	E1	5681-5682	Exon 18+19+20+21 Multiplex Dispense Ready	5670-5671
	G719 Multiplex Ready-to-use	E2	2081-2082	G719 Multiplex Dispense Ready	2085-2086
	G719 Simplex Ready-to-use	E3	3071-3072	G719 Simplex Dispense Ready	3027-3028
	Del 19 Multiplex Ready-to-use	E4	2071-2072	Del 19 Multiplex Dispense Ready	2075-2076
	S768I Simplex Ready-to-use	E5	2091-2092	S768I Simplex Dispense Ready	2095-2096
	T790M Simplex Ready-to-use	E6	2061-2062	T790M Simplex Dispense Ready	2065-2066
	Exon 20 Insertions Multiplex Ready-to-use	E7	3021-3022	Ex20Ins Multiplex Dispense Ready	3025-3026
	L858R Simplex Ready-to-use	E8	3001-3002	L858R Simplex Dispense Ready	3005-3006
	L861Q Simplex Ready-to-use	E9	3010-3012	L861Q Simplex Dispense Ready	3015-3016
	Del 19 Multiplex, T790M, L858R Ready-to-use	E10	5398-5399	Del 19 Multiplex, T790M, L858R Dispense Ready	3077-3078
<b>KIT*</b>	D816V Simplex Ready-to-use	I1	3031-3032	D816V Simplex Dispense Ready	3035-3036
<b>KRAS</b>	Exon 2+3+4 Multiplex Ready-to-use	K1	1701-1702	Exon 2+3+4 Multiplex Dispense Ready	1900-1901
	Exon 2 Multiplex Ready-to-use	K2	1706-1707	Exon 2 Multiplex Dispense Ready	1905-1906
	Exon 3 Multiplex Ready-to-use	K3	1711-1712	Exon 3 Multiplex Dispense Ready	1910-1911
	Exon 4 Multiplex Ready-to-use	K4	1716-1717	Exon 4 Multiplex Dispense Ready	1915-1916
	Exon 2 Simplex Ready-to-use	K5+K6	1721-1722	Exon 2 Simplex Dispense Ready	1920-1921
	Exon 2 Simplex Ready-to-use (G12R, G12C, G12S and G12V)	K5	1726-1727	Exon 2 Simplex A Dispense Ready (G12R, G12C, G12S and G12V)	1925-1926
	Exon 2 Simplex Ready-to-use (G12A, G12D and G13D)	K6	1731-1732	Exon 2 Simplex B Dispense Ready (G12A, G12D and G13D)	1930-1931
	Exon 3 Simplex Ready-to-use	K7+K8	1736-1737	Exon 3 Simplex Dispense Ready	1935-1936
	Exon 3 Simplex Ready-to-use (Q61H1, Q61K, Q61L and A59T)	K7	1741-1742	Exon 3 Simplex A Dispense Ready (Q61H1, Q61K, Q61L and A59T)	1940-1941
	Exon 3 Simplex Ready-to-use (Q61H2, Q61E, Q61R and A59G)	K8	1746-1747	Exon 3 Simplex B Dispense Ready (Q61H2, Q61E, Q61R and A59G)	1945-1946
	Exon 4 Simplex Ready-to-use	K9+K10	1751-1752	Exon 4 Simplex Dispense Ready	1950-1951
	Exon 4 Simplex Ready-to-use (K117N1 and K117N2)	K9	1756-1757	Exon 4 Simplex Dispense Ready (K117N1 and K117N2)	1955-1956
	Exon 4 Simplex Ready-to-use (A146P, A146T and A146V)	K10	1761-1762	Exon 4 Simplex Dispense Ready (A146P, A146T and A146V)	1960-1961
<b>NRAS</b>	Exon 2+3+4 Multiplex Ready-to-use	N1	1771-1772	Exon 2+3+4 Dispense Ready	2000-2001
	Exon 2 Multiplex Ready-to-us	N2	1776-1777	Exon 2 Multiplex Dispense Ready	2005-2006
	Exon 3 Multiplex Ready-to-use	N3	1781-1782	Exon 3 Multiplex Dispense Ready	2010-2011
	Exon 4 Multiplex Ready-to-use	N4	1786-1787	Exon 4 Multiplex Dispense Ready	2015-2016
	Exon 2 Simplex Ready-to-use	N5+N6	1791-1792	Exon 2 Simplex Dispense Ready	2020-2021

	Exon 2 Simplex Ready-to-use (G12A, G12C, G12D, G12R, G12S and G12V)	N5	1796-1797	Exon 2 Simplex Dispense Ready (G12A, G12C, G12D, G12R, G12S and G12V)	2025-2026
	Exon 2 Simplex Ready-to-use (G13A, G13C, G13D, G13R, G13S and G13V)	N6	1801-1802	Exon 2 Simplex Dispense Ready (G13A, G13C, G13D, G13R, G13S and G13V)	2030-2031
	Exon 3 Simplex Ready-to-use	N7+N8	1806-1807	Exon 3 Simplex Dispense Ready	2035-2036
	Exon 3 Simplex Ready-to-use (Q61H1, Q61H2, Q61K, Q61L and Q61R)	N7	1811-1812	Exon 3 Simplex A Dispense Ready (Q61H1, Q61H2, Q61K, Q61L and Q61R)	2040-2041
	Exon 3 Simplex Ready-to-use (A59D and A59T)	N8	1816-1817	Exon 3 Simplex B Dispense Ready (A59D and A59T)	2045-2046
	Exon 4 Simplex Ready-to-use	N9+N10	5356-5357	Exon 4 Simplex Dispense Ready	2048-2049
	Exon 4 Simplex Ready-to-use (N117N1 and N117N2)	N9	1821-1822	Exon 4 Simplex Dispense Ready (N117N1 and N117N2)	2050-2051
	Exon 4 Simplex Ready-to-use (A146P, A146T and A146V)	N10	1826-1827	Exon 4 Simplex Dispense Ready (A146P, A146T and A146V)	2055-2056
<b>PIK3CA*</b>	PIK3CA Multiplex Ready-to-use	P1	3041-3042	PIK3CA Multiplex Dispense Ready	3045-3046
	PIK3CA Simplex Ready-to-use (H1047R, H1047Y and H1047L)	P2	3051-3052	PIK3CA Simplex Dispense Ready (H1047R, H1047Y and H1047L)	3055-3056

\* Research use only

### 13. ADDITIONAL INFORMATION

SensiScreen® is CE IVD labeled medical equipment intended for *in vitro* diagnostic in compliance with EU Directive 98/79/EC. SensiScreen® is a Class I non-invasive device according to EU directive 93/42/EEC. TaqMan® is a trademark of Roche. 5-FAM™, VIC®, TET™ and HEX™ are trademarks and registered trademarks of Applied Biosystems or its subsidiaries in the U.S. and certain other countries. Inc. SensiScreen®, 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.

A patent application of SensiScreen® has been submitted. Some parts of the assays are already covered by the granted patent WO2007104318 A3.

© Copyright 2016. 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

#### 13.1 DATE OF REVISION

SensiScreen® protocol was revised February 2019.

#### Change history

Version No.	Effective Date	Significant Changes	Previous version
3.0	Beta version	New Procedure	N/A
3.1	Beta version	Addition of EGFR G719 simplex assays	3.0
3.11	Beta version	EGFR and KIT product codes has been revised	3.1
3.12	Released May 2017	Page 7 E8 multiplex has been corrected to simplex and BRAF cross signal has been removed	3.11
3.13	July 2017	Front page layout changed. Another reference added to the list on page 18.	3.12

		Three EGFR combination products (product codes) has been added to the table on page 20	
3.14	September 2017	Added EGFR exon 20 insertions, KIT and PIK3CA assays in table 1. Renamed EGFR assays and reorganized table 2, table 3 and product overview table (section 12).	3.13
3.15	December 2017	Changed configuration of EGFR multiplex (E1) assay. Added Myd88 to product overview.	3.14
3.16	January 2018	Added EGFR Exon 19 Deletions; T790M; L858R assay (strip E10)	3.15
3.21	January 2018	Revised sections 1, 2, 3.2, 4.2-4.5, 5, 7.2, 8, 10 and 11 including figures (1+2) and tables (2-3, 6-8).  Changed the nomenclature of the references in table 2+3  Added guidelines about baseline correction and internal control analysis (section 8)	3.16
3.22	January 2018	Changed the Cat. no. of EGFR Strip E10	3.21
3.23	February 2018	Added the Mic Real Time PCR cyclers to validated real time PR instruments	3.22
3.24	June 2018	Removed MYD88 from the list of included assays. MYD88 is as of this date a PlentiPlex™ assay	3.23
3.3	February 2019	Updated product overview table	3.24

## 14. NOTES

---