Sensi creen®

Made by PentaBase

INSTRUCTIONS FOR USE
SENSISCREEN® FFPE ASSAYS

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

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CE IVD

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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.

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.1799_1800TG>AA	p.Val600Glu (V600E)	COSM475
	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 deletions	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 substitutions	c.2369C>T	p.Thr790Met (T790M)	COSM6240	
	c.2303G>T	p.Ser768Ile	COSM6241	
EGFR exon 20 insertions	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_2311insGGCACA	p.D770_N771insGT	COSM1238029	Multiplex 1
	c.2310_2311insGGGTTT	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_2321insCCCACG	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 c.2573_2574TG>GT	p.Leu858Arg p.Leu858Arg	COSM6224 COSM12429	
	c.2582T>A	p.Leu861Gln	COSM6213	

KIT mutations detected with SensiScreen®

Assay	CDS mutation	Amino acid substitution	Cosmic ID
KIT D816V	c.2447A>T	Asp816Val	COSM1314

KRAS mutations detected with SensiScreen®

Assay	CDS mutation	Amino acid substitution	Cosmic ID
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

NRAS mutations detected with SensiScreen®

Assay	CDS mutation	Amino acid substitution	Cosmic ID
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.176C>A	p.Ala59Asp (A59D)	COSM253327
	c.175G>A	p.Ala59Thr (A59T)	COSM578
	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

PIK3CA mutations detected with SensiScreen®

Assay	CDS mutation	Amino acid substitution	Cosmic ID
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™ (λ_{abs}. 495 nm and λ_{Em}. 516 nm, detected on the same channel as FAM™) in combination with Green Quencher™, or as PentaYellow™ (λ_{abs}. 533 nm and λ_{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.

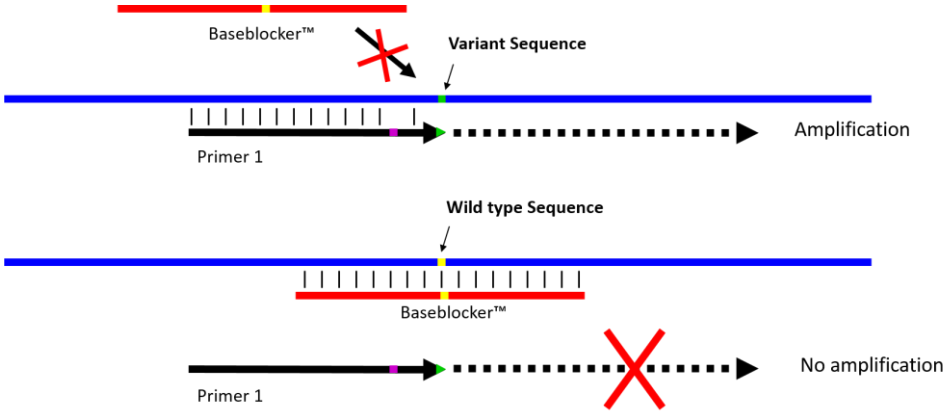

Figure 1: Illustration of how BaseBlockers™ function in SensiScreen® assays. A BaseBlocker™ binds to and blocks the wild type template from being amplified. In contrast, the BaseBlocker™ does not inhibit amplification of a template with a single nucleotide mutation and the result is a selective amplification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

List of SensiScreen® FFPE Ready-to-use assays

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

		B	EGFR L858R Simplex	L858R	EGFR Reference 6
E9	**EGFR L861Q**	**A**	**EGFR Reference 7**		
		B	EGFR L861Q Simplex	L861Q	EGFR Reference 7
E10	**EGFR exon 19 Deletions; T790M; L858R**	**A**	**EGFR Reference 4**		
		B	EGFR exon 19 Deletions	35 deletions. See table1.	EGFR Reference 4
		C	EGFR T790M Simplex	T790M	EGFR Reference 4
		D	EGFR L858R Simplex	L858R	EGFR Reference 4

KIT D816V Assay

Strip #	Gene	Tube #	Content	Mutations	Corresponding reference
I1*	**KIT**	**A**	**KIT Reference 1**		
		B	KIT Simplex 1	D816V	KIT Reference 1

KRAS Assays

Strip #	Gene	Tube #	Content	Mutations	Corresponding reference
K1	**KRAS exon 2+3+4 Multiplex**	**A**	**KRAS Reference 1**		
		B	KRAS exon 2 Multiplex 1	G12R; G12C; G12S; G12V	KRAS Reference 1
		C	KRAS exon 2 Multiplex 2	G12A; G12D; G13D	KRAS Reference 1
		D	**KRAS Reference 3**		
		E	KRAS exon 3 Multiplex 1	Q61H1; Q61K; Q61L; A59T	KRAS Reference 3
		F	KRAS exon 3 Multiplex 2	Q61H2; Q61E; Q61R; A59G	KRAS Reference 3
		G	KRAS exon 4 Multiplex 1	K117N; K117N2	KRAS Reference 3
		H	KRAS exon 4 Multiplex 2	A146P; A146T; A146V	KRAS Reference 3
K2	**KRAS exon 2 Multiplex**	**A**	**KRAS Reference 1**		
		B	KRAS exon 2 Multiplex 1	G12R; G12C; G12S; G12V	KRAS Reference 1
		C	KRAS exon 2 Multiplex 2	G12A; G12D; G13D	KRAS Reference 1
K3	**KRAS exon 3 Multiplex**	**A**	**KRAS Reference 2**		
		B	KRAS exon 3 Multiplex 1	Q61H1; Q61K; Q61L; A59T	KRAS Reference 2
		C	KRAS exon 3 Multiplex 2	Q61H2; Q61E; Q61R; A59G	KRAS Reference 2
K4	**KRAS exon 4 Multiplex**	**A**	**KRAS Reference 3**		
		B	KRAS exon 4 Multiplex 1	K117N; K117N2	KRAS Reference 3
		C	KRAS exon 4 Multiplex 2	A146P; A146T; A146V	KRAS Reference 3
K5	**KRAS exon 2 Simplex 1**	**A**	**KRAS Reference 1**		
		B	KRAS exon 2 G12R Simplex	G12R	KRAS Reference 1
		C	KRAS exon 2 G12C Simplex	G12C	KRAS Reference 1
		D	KRAS exon 2 G12S Simplex	G12S	KRAS Reference 1
		E	KRAS exon 2 G12V Simplex	G12V	KRAS Reference 1
K6	**KRAS exon 2 Simplex 2**	**A**	**KRAS Reference 1**		
		B	KRAS exon 2 G12A Simplex	G12A	KRAS Reference 1
		C	KRAS exon 2 G12D Simplex	G12D	KRAS Reference 1
		D	KRAS exon 2 G13D Simplex	G13D	KRAS Reference 1
K7	**KRAS exon 3 Simplex 1**	**A**	**KRAS Reference 2**		
		B	KRAS exon 3 Q61H1 Simplex	Q61H1	KRAS Reference 2
		C	KRAS exon 3 Q61K Simplex	Q61K	KRAS Reference 2
		D	KRAS exon 3 Q61L Simplex	Q61L	KRAS Reference 2
		E	KRAS exon 3 A59T Simplex	A59T	KRAS Reference 2
K8	**KRAS exon 3 Simplex 2**	**A**	**KRAS Reference 2**		
		B	KRAS exon 3 Q61H2 Simplex	Q61H2	KRAS Reference 2
		C	KRAS exon 3 Q61E Simplex	Q61E	KRAS Reference 2
		D	KRAS exon 3 Q61R Simplex	Q61R	KRAS Reference 2
		E	KRAS exon 3 A59G Simplex	A59G	KRAS Reference 2
K9	**KRAS exon 4 Simplex 1**	**A**	**KRAS Reference 3**		
		B	KRAS exon 4 K117N1 Simplex	K117N1	KRAS Reference 3
		C	KRAS exon 4 K117N2 Simplex	K117N2	KRAS Reference 3
K10	**KRAS exon 4 Simplex 2**	**A**	**KRAS Reference 4**		
		B	KRAS exon 4 A146P Simplex	A146P	KRAS Reference 4
		C	KRAS exon 4 A146T Simplex	A146T	KRAS Reference 4
		D	KRAS exon 4 A146V Simplex	A146V	KRAS Reference 4

NRAS Assays

Strip #	Gene	Tube #	Content	Mutations	Corresponding reference
N1	**NRAS exon 2+3+4 Multiplex**	**A**	**NRAS Reference 1**		
		B	NRAS exon 2 Multiplex 1	G12A; G12C; G12D; G12R; G12S; G12V	NRAS Reference 1
		C	NRAS exon 2 Multiplex 2	G13A; G13C; G13D; G13R; G13S; G13V	NRAS Reference 1
		D	**NRAS Reference 2**		
		E	NRAS exon 3 Multiplex 1	Q61H1; Q61H2; Q61K; Q61L; Q61R	NRAS Reference 2
		F	NRAS exon 3 Multiplex 2	A59D; A59T	NRAS Reference 2
		G	NRAS exon 4 Multiplex 1	K117N1; K117N2	NRAS Reference 2
		H	NRAS exon 4 Multiplex 1	A146P; A146T; A146V	NRAS Reference 2
N2	**NRAS exon 2 Multiplex**	**A**	**NRAS Reference 1**		
		B	NRAS exon 2 Multiplex 1	G12A; G12C; G12D; G12R; G12S; G12V	NRAS Reference 1
		C	NRAS exon 2 Multiplex 2	G13A; G13C; G13D; G13R; G13S; G13V	NRAS Reference 1
N3	**NRAS exon 3 Multiplex**	**A**	**NRAS Reference 2**		
		B	NRAS exon 3 Multiplex 1	Q61H1; Q61H2; Q61K; Q61L; Q61R	NRAS Reference 2

		C	NRAS exon 3 Multiplex 2	A59D; A59T	NRAS Reference 2
N4	NRAS exon 4 Multiplex	A	**NRAS Reference 3**		
		B	NRAS exon 4 Multiplex 1	K117N1; K117N2	NRAS Reference 3
		C	NRAS exon 4 Multiplex 2	A146P; A146T; A146V	NRAS Reference 3
N5	NRAS exon 2 Simplex 1	A	**Reference 1**		
		B	NRAS exon 2 G12A Simplex	G12A	NRAS Reference 1
		C	NRAS exon 2 G12C Simplex	G12C	NRAS Reference 1
		D	NRAS exon 2 G12D Simplex	G12D	NRAS Reference 1
		E	NRAS exon 2 G12R Simplex	G12R	NRAS Reference 1
		F	NRAS exon 2 G12S Simplex	G12S	NRAS Reference 1
		G	NRAS exon 2 G12V Simplex	G12V	NRAS Reference 1
N6	NRAS exon 2 Simplex 2	A	**NRAS Reference 4**		
		B	NRAS exon 2 G13A Simplex	G13A	NRAS Reference 4
		C	NRAS exon 2 G13C Simplex	G13C	NRAS Reference 4
		D	NRAS exon 2 G13D Simplex	G13D	NRAS Reference 4
		E	NRAS exon 2 G13R Simplex	G13R	NRAS Reference 4
		F	NRAS exon 2 G13S Simplex	G13S	NRAS Reference 4
		G	NRAS exon 2 G13V Simplex	G13V	NRAS Reference 4
N7	NRAS exon 3 Simplex 1	A	**NRAS Reference 2**		
		B	NRAS exon 3 Q61H1 Simplex	Q61H1	NRAS Reference 2
		C	NRAS exon 3 Q61H2 Simplex	Q61H2	NRAS Reference 2
		D	NRAS exon 3 Q61K Simplex	Q61K	NRAS Reference 2
		E	NRAS exon 3 Q61L Simplex	Q61L	NRAS Reference 2
		F	NRAS exon 3 Q61R Simplex	Q61R	NRAS Reference 2
N8	NRAS exon 3 Simplex 2	A	**NRAS Reference 2**		
		B	NRAS exon 3 A59D Simplex	A59D	NRAS Reference 2
		C	NRAS exon 3 A59T Simplex	A59T	NRAS Reference 2
N9	NRAS exon 4 Simplex 1	A	**NRAS Reference 3**		
		B	NRAS exon 4 K117N1 Simplex	K117N1	NRAS Reference 3
		C	NRAS exon 4 K117N2 Simplex	K117N2	NRAS Reference 3
N10	NRAS exon 4 Simplex 2	A	**NRAS Reference 5**		
		B	NRAS exon 4 A146P Simplex	A146P	NRAS Reference 5
		C	NRAS exon 4 A146T Simplex	A146T	NRAS Reference 5
		D	NRAS exon 4 A146V Simplex	A146V	NRAS Reference 5

PIK3CA H1047 Assays

Strip #	Gene	Tube #	Content	Mutations	Corresponding reference
P1*	PIK3CA Multiplex	A	**PIK3CA Reference 1**		
		B	PIK3CA Multiplex	H1047R; H1047Y, H1047L	PIK3CA Reference 1
P2*	PIK3CA Simplex	A	**PIK3CA Reference 1**		
		B	PIK3CA H1047L Simplex	H1047L	PIK3CA Reference 1
		C	PIK3CA H1047R Simplex	H1047R	PIK3CA Reference 1
		D	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.

List of SensiScreen FFPE Dispense-Ready assays

Gene	Tube #	Content	Mutations	Corresponding reference	Volume 20x	Volume 50x
BRAF V600 Multiplex	1	**BRAF Reference 1**			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
BRAF V600 Simplex	1	**BRAF Reference 1**			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
BRAF V600E Simplex	1	**BRAF Reference 1**			150 µL	375 µL
	2	BRAF V600E Simplex	V600E	BRAF Reference 1	150 µL	375 µL
	3	Mastermix			500 µL	1250 µL

EGFR Assays

Gene	Tube #	Content	Mutations	Corresponding reference	Volume 20x	Volume 50x
EGFR Exon 18+19+20+21	1	**EGFR Reference 1**			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
EGFR G719 Multiplex	1	**EGFR Reference 1**			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	**EGFR Reference 1**			150 µL	375 µL
	2	EGFR exon 18 G719A Simplex	G719A	EGFR Reference 1	150 µL	375 µL
	3	EGFR exon 18 G719C Simplex	G719C	EGFR Reference 1	150 µL	375 µL
	4	EGFR exon 18 G719S Simplex	G719S	EGFR Reference 1	150 µL	375 µL
	5-6	Mastermix			1000 µL	2500 µL
EGFR Exon 19 Deletions	1	**EGFR Reference 2**			150 µL	375 µL
	2	EGFR exon 19 Multiplex	35 deletions. See table 1.	EGFR Reference 2	150 µL	375 µL
	3	Mastermix			500 µL	1250 µL
EGFR S768I	1	**EGFR Reference 3**			150 µL	375 µL
	2	EGFR S768I Simplex	S768I	EGFR Reference 3	150 µL	375 µL
	3	Mastermix			500 µL	1250 µL
EGFR T790M	1	**EGFR Reference 4**			150 µL	375 µL
	2	EGFR T790M Simplex	T790M	EGFR Reference 4	150 µL	375 µL
	3	Mastermix			500 µL	1250 µL
EGFR Exon 20 Insertions	1	**EGFR Reference 5**			150 µL	375 µL
	2	EGFR exon 20 Multiplex 1	13 insertions. See table 1.	EGFR Reference 5	150 µL	375 µL
	3	EGFR exon 20 Multiplex 2	9 insertions. See table 1.	EGFR Reference 5	150 µL	375 µL
	4	Mastermix			750 µL	1875 µL
EGFR L858R	1	**EGFR Reference 6**			150 µL	375 µL
	2	EGFR L858R Simplex	L858R	EGFR Reference 6	150 µL	375 µL
	3	Mastermix			500 µL	1250 µL
EGFR L861Q	1	**EGFR Reference 7**			150 µL	375 µL
	2	EGFR L861Q Simplex	L861Q	EGFR Reference 7	150 µL	375 µL
	3	Mastermix			500 µL	1250 µL
EGFR exon 19 deletions; T790M; L858R	1	**EGFR Reference 4**			150 µL	375 µL
	2	EGFR exon 19 Deletions	35 deletions. See table below.	EGFR Reference 4	150 µL	375 µL
	3	EGFR T790M Simplex	T790M	EGFR Reference 4	150 µL	375 µL
	4	EGFR L858R Simplex	L858R	EGFR Reference 4	150 µL	375 µL
	5-6	Mastermix			1000 µL	2500 µL

KIT D816V Assay

Gene	Tube #	Content	Mutations	Corresponding reference	Volume 20x	Volume 50x
KIT D816V*	1	**KIT Reference 1**			150 µL	375 µL
	2	KIT Simplex 1	D816V	KIT Reference 1	150 µL	375 µL
	3	Mastermix			500 µL	1250 µL

KRAS Assays

Gene	Tube #	Content	Mutations	Corresponding reference	Volume 20x	Volume 50x
KRAS exon 2+3+4 Multiplex	1	**KRAS Reference 1**			150 µL	375 µL
	2	KRAS exon 2 Multiplex 1	G12R; G12C; G12S; G12V	KRAS Reference 1	150 µL	375 µL
	3	KRAS exon 2 Multiplex 2	G12A; G12D; G13D	KRAS Reference 1	150 µL	375 µL
	4	**KRAS Reference 3**			160 µL	375 µL
	5	KRAS exon 3 Multiplex 1	Q61H1; Q61K; Q61L; A59T	KRAS Reference 3	150 µL	375 µL
	6	KRAS exon 3 Multiplex 2	Q61H2; Q61E; Q61R; A59G	KRAS Reference 3	150 µL	375 µL
	7	KRAS exon 4 Multiplex 1	K117N; K117N2	KRAS Reference 3	150 µL	375 µL
	8	KRAS exon 4 Multiplex 2	A146P; A146T; A146V	KRAS Reference 3	150 µL	375 µL
	9-11	Mastermix			2000 µL	5000 µL
KRAS exon 2 Multiplex	1	**KRAS Reference 1**			150 µL	375 µL
	2	KRAS exon 2 Multiplex 1	G12R; G12C; G12S; G12V	KRAS Reference 1	150 µL	375 µL
	3	KRAS exon 2 Multiplex 2	G12A; G12D; G13 D	KRAS Reference 1	150 µL	375 µL
	4	Mastermix			750 µL	1875 µL
KRAS exon 3 Multiplex	1	**KRAS Reference 2**			150 µL	375 µL
	2	KRAS exon 3 Multiplex 1	Q61H1; Q61K; Q61L; A59T	KRAS Reference 2	150 µL	375 µL
	3	KRAS exon 3 Multiplex 2	Q61H2; Q61E; Q61R; A59G	KRAS Reference 2	150 µL	375 µL
	4	Mastermix			750 µL	1875 µL
KRAS exon 4 Multiplex	1	**KRAS Reference 3**			150 µL	375 µL
	2	KRAS exon 4 Multiplex 1	K117N; K117N2	KRAS Reference 2	150 µL	375 µL
	3	KRAS exon 4 Multiplex 2	A146P; A146T; A146V	KRAS Reference 2	150 µL	375 µL
	4	Mastermix			750 µL	1875 µL
KRAS exon 2 Simplex 1	1	**KRAS Reference 1**			150 µL	375 µL
	2	KRAS exon 2 G12R Simplex	G12R	KRAS Reference 1	150 µL	375 µL
	3	KRAS exon 2 G12C Simplex	G12C	KRAS Reference 1	150 µL	375 µL
	4	KRAS exon 2 G12S Simplex	G12S	KRAS Reference 1	150 µL	375 µL
	5	KRAS exon 2 G12V Simplex	G12V	KRAS Reference 1	150 µL	375 µL
	6-7	Mastermix			1250 µL	3125 µL
KRAS exon 2 Simplex 2	1	**KRAS Reference 1**			150 µL	375 µL
	2	KRAS exon 2 G12A Simplex	G12A	KRAS Reference 1	150 µL	375 µL

	3	KRAS exon 2 G12D Simplex	G12D	KRAS Reference 1	150 µL	375 µL
	4	KRAS exon 2 G13D Simplex	G13D	KRAS Reference 1	150 µL	375 µL
	5-6	Mastermix			1000 µL	2500 µL
KRAS exon 3 Simplex 1	1	**KRAS Reference 2**			150 µL	375 µL
	2	KRAS exon 3 Q61H1 Simplex	Q61H1	KRAS Reference 2	150 µL	375 µL
	3	KRAS exon 3 Q61K Simplex	Q61K	KRAS Reference 2	150 µL	375 µL
	4	KRAS exon 3 Q61L Simplex	Q61L	KRAS Reference 2	150 µL	375 µL
	5	KRAS exon 3 A59T Simplex	A59T	KRAS Reference 2	150 µL	375 µL
	6-7	Mastermix			1250 µL	3125 µL
KRAS exon 3 Simplex 2	1	**KRAS Reference 2**			150 µL	375 µL
	2	KRAS exon 3 Q61H2 Simplex	Q61H2	KRAS Reference 2	150 µL	375 µL
	3	KRAS exon 3 Q61E Simplex	Q61E	KRAS Reference 2	150 µL	375 µL
	4	KRAS exon 3 Q61R Simplex	Q61R	KRAS Reference 2	150 µL	375 µL
	5	KRAS exon 3 A59G Simplex	A59G	KRAS Reference 2	150 µL	375 µL
	6-7	Mastermix			1250 µL	3125 µL
KRAS exon 4 Simplex 1	1	**KRAS Reference 3**			150 µL	375 µL
	2	KRAS exon 4 K117N1 Simplex	K117N	KRAS Reference 3	150 µL	375 µL
	3	KRAS exon 4 K117N2 Simplex	K117N2	KRAS Reference 3	150 µL	375 µL
	4	Mastermix			750 µL	1875 µL
KRAS exon 4 Simplex 2	1	**KRAS Reference 4**			150 µL	375 µL
	2	KRAS exon 4 A146P Simplex	A146P	KRAS Reference 4	150 µL	375 µL
	3	KRAS exon 4 A146T Simplex	A146T	KRAS Reference 4	150 µL	375 µL
	4	KRAS exon 4 A146V Simplex	A146V	KRAS Reference 4	150 µL	375 µL
	5-6	Mastermix			1000 µL	2500 µL

NRAS Assays

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

NRAS exon 4 Simplex 1	1	**NRAS Reference 3**			150 µL	375 µL
	2	NRAS exon 4 K117N1 Simplex	K117N1	NRAS Reference 3	150 µL	375 µL
	3	NRAS exon 4 K117N2 Simplex	K117N2	NRAS Reference 3	150 µL	375 µL
	4	Mastermix			750 µL	1875 µL
NRAS exon 4 Simplex 2	1	**NRAS Reference 5**			150 µL	375 µL
	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
PIK3CA H1047 Assays						
Gene	**Tube #**	**Content**	**Mutations**	**Corresponding reference**	**Volume 20x**	**Volume 50x**
PIK3CA Multiplex*	1	**PIK3CA Reference 1**			150 µL	375 µL
	2	PIK3CA Multiplex	H1047L, H1047R, H1047Y	PIK3CA Reference 1	150 µL	375 µL
	3	Mastermix			300 µL	1250 µL
PIK3CA Simplex*	1	**PIK3CA Reference 1**			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 SensiScreen®

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_2O (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, StepOne, QuantStudio 5 and 12, and PRISM®7900HT (Applied Biosystems), Mic (bio molecular systems), Gentier 48 and 96 (XI'AN TIANLONG SCIENCE AND TECHNOLOGY CO.) and Lightcycler® 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 or super-cooled ice bricks (-80°C), 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 A/S (see section 11 “Manufacturer and Distributors”). Please also contact your local distributor or PentaBase A/S 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 genomic DNA should be stored at maximum -15˚C until use.

6.1 Recommended procedure for extraction of genomic DNA

There are no specific requirements to the methods used for extraction of genomic DNA for use with SensiScreen® assays. It is however recommended to use methods and/or procedures intended for extraction of genomic DNA from the specimen of interest that removes PCR inhibitors embedded in the sample such as formalin and paraffin. Thus, for extraction of genomic DNA from FFPE samples, it is recommended to use genomic DNA extraction kits and/or procedures specially designed for handling of FFPE samples including steps for deparaffinization and sample digestion. Regardless of method used, it is recommended to follow the manufacturers protocol for genomic DNA 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 µL extracted gDNA (1-10 ng/µL) 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 Simplex 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)
	60˚C	60 sec		HEX™/VIC™/TET™ (538 nm/551 nm)
				Measure fluorescence intensity at the end of each cycle

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 ΔCt 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 ΔCt 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 ΔCt values are within the specified range for each mutation.

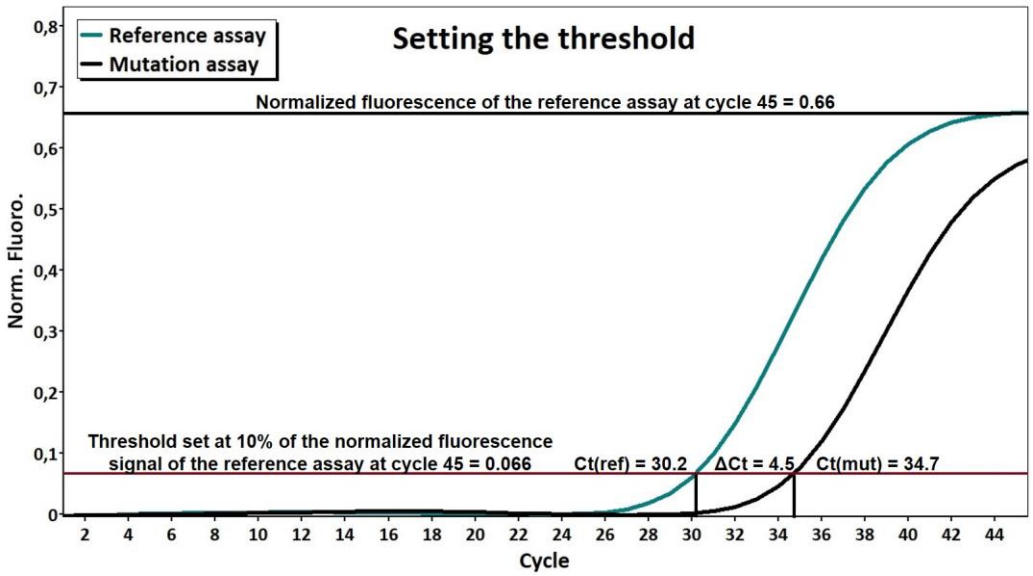

Figure 2: Setting the threshold. Read the fluorescence value for the reference assay at cycle 45 and set the threshold at 10% of this value. This setting for the threshold is now used for the analysis of the corresponding mutation assay(s). In the shown example, ΔCt = Ct(mut)-Ct(ref) = 34.7-30.2 = 4.5. Thus, the sample is positive for the mutation analyzed.

8.3 Analysis protocol

Use the following protocol to determine the mutational status:

1. 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 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.

7. Calculate ΔCt 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 ΔCt is equal to or below 9 and negative if the ΔCt is above 9 cf. Table 8.

ΔCt for assay	Conclusion	Comments
ΔCt ≤9	Positive	The sample is mutation positive if ΔCt ≤9 and Ct ≤39
ΔCt >9	Negative	The sample is mutation negative if ΔCt ≥9 or Ct ≤39

Table 8: Mutation analysis

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. Non-clinical performance

9.1 Analytical sensitivity – Limit of blank

The non-clinical specificity and performance of SensiScreen® FFPE assays in the absence of template has been established and evaluated during assay validation and is evaluated during quality control of produced lots using 50 ng of wild-type human genomic DNA and PCR grade water, respectively. The criteria for approval of assays are $Ct_{(50\ ng\ WT)} > 40$ or $\Delta Ct_{(50\ ng\ WT)} > 12.5$ and $Ct_{(NTC)} > 45$.

9.2 Analytical sensitivity – Limit of detection

The non-clinical limit of detection (LOD) of SensiScreen® FFPE assays has been evaluated using serial dilutions of plasmid or cell line mutated DNA in a 50 ng wild type DNA background.

9.2.1 Limit of detection BRAF

SensiScreen® BRAF FFPE Limit of detection

Gene	CDS mutation	Amino acid substitution	Cosmic ID	SensiScreen® Simplex			SensiScreen® Multiplex		
				LOD	Assay#	Cat. No.	LOD	Assay#	Cat. No.
BRAF exon 15	c.1799_1800TG>AT	p.Val600Asp (V600D)	COSM477	0.7%	B2	1402-1403 1836-1837	0.5%	B1	1398-1399 1831-1832
	c.1799T>A	p.Val600Glu (V600E1)	COSM476	0.6%	B2 B3	1400-1401 1402-1403 1836-1837 1841-1842	0.6%		
	c.1799_1800TG>AA	p.Val600Glu (V600E2)	COSM475	ND	B2 B3	1400-1401 1402-1403 1836-1837 1841-1842	ND		
	c.1798_1799GT>AA	p.Val600Lys (V600K)	COSM473	0.9%	B2	1402-1403 1836-1837	0.7%		
	c.1798_1799GT>AG	p.Val600Arg (V600R)	COSM474	1.3%	B2	1402-1403 1836-1837	1.7%		

Table 9: Sensitivity of SensiScreen® FFPE BRAF assays. Plasmid or cell-line DNA harboring the indicated BRAF Mutations were serially diluted in 50 ng wild type human genomic DNA and analyzed with SensiScreen® BRAF Simplex and Multiplex assays using MyGo Pro real-time PCR instruments (IT-IS Life Science). Limit of detection (LOD, % mutation) was calculated using the formula 9 = A ln(x) + B where A is the slope of the logarithmic trendline (R^2>98) of at least three of the serial dilution points 10%, 5%, 2%, 1% and 0.5%, and B is the value (ΔCt) at 1% mutation. ND: Not determined

9.2.2 LIMIT OF DETECTION EGFR

SensiScreen® EGFR FFPE Limit of detection									
Gene	**CDS mutation**	**Amino acid substitution**	**Cosmic ID**	**SensiScreen® Simplex**			**SensiScreen® Multiplex**		
				LOD	**Assay#**	**Cat. No.**	**LOD**	**Assay#**	**Cat. No.**
EGFR exon 18	c.2156G>C	p.Gly719Ala (G719A)	COSM6239	1.4%	E3	3027-3028 3071-3072	1.2%	E1 E2	2081-2082 2085-2086 5681-5682 5670-5671
	c.2155G>T	p.Gly719Cys (G719C)	COSM6253	0.8%			0.7%		
	c.2155G>A	p.Gly719Ser (G719S)	COSM6252	0.5%			0.7%		
EGFR exon 19 deletions	c.2240_2251del12	p.L747_T751>S	COSM6210	NA			<1%*	2071-2072 2075-2076 E1 E4 E10	3077-3078 5398-5399 5670-5671 5681-5682
	c.2239_2247del9	p.L747_E749delLRE	COSM6218						
	c.2238_2255del18	p.E746_S752>D	COSM6220						
	c.2235_2249del15	p.E746_A750delELREA	COSM6223				0.2%		
	c.2236_2250del15	p.E746_A750delELREA	COSM6225				<1%*		
	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_2248TTAAGAGAAG>C	p.L747_A750>P	COSM12382				0.2%		
	c.2239_2251>C (complex)	p.L747_T751>P	COSM12383				<1%*		
	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 subst.	c.2369C>T	p.Thr790Met (T790M)	COSM6240	0.5%	E1 E6 E10	2061-2062 2065-2066 3077-3078	NA		

						5398-5399 5670-5671 5681-5682			
	c.2303G>T	p.Ser768Ile (S768I)	COSM6241	0.6%		2091-2092 2095-2096	0.5%	E1	5681-5682 5670-5671
EGFR exon 20 insertions	c.2300_2301insCAGCGTGGA	p.D770_N771insSVD	COSM3728433	NA			<1%^	E1 E7	3021-3022 3025-3026 5670-5670 5681-5682
	c.2302_2303insCGCTGGCCA	p.A767_S768insTLA	COSM12425						
	c.2307_2308ins15	p.V769_D770insMASVD	COSM28638						
	c.2307_2308insGCCAGCGTG	p.V769_D770insASV	COSM12376						
	c.2308_2309insCCAGCGTGG	p.V769_D770insASV	COSM12426				0.7%		
	c.2308_2309insGGGTCGTGG	p.V769_D770insGVV	COSM18430				<1%^		
	c.2308_2309insGTT	p.D770>GY	COSM12427						
	c.2309_2310AC>CCAGCGTGGAT	p.V769_D770insASV	COSM13558						
	c.2310_2311insAGCGTGGAC	p.D770_N771insSVD	COSM85749						
	c.2310_2311insGGCACA	p.D770_N771insGT	COSM1238029						
	c.2310_2311insGGGTTT	p.D770_N771insGF	COSM655155						
	c.2310_2311insGGT	p.D770_N771insG	COSM12378				0.3%		
	c.2310_2311insAACCCCCAC	p.H773_V774insNPH	COSM48920				<1%^		
	c.2310_2311ins9GCGTGGACA	p.D770_N771insSVD	COSM13428						
	c.2316_2317insNNN	p.P772_H773insX	COSM21597						
	c.2319_2320insAACCCCCAC	p.H773_V774insNPH	COSM12381						
	c.2319_2320insCAC	p.H773_V774insH	COSM12377				0.4%		
	c.2319_2320insCCCCAC	p.H773_V774insPH	COSM12380				<1%^		
	c.2320_2321insCCCACG	p.H773_V774insAH	COSM1238028						
	c.2321_2322insCCACGT	p.V774_C775insHV	COSM18432						
	c.2322_2323insCACGTG	p.V774_C775insHV	COSM22948						
EGFR exon 21	c.2573T>G	p.Leu858Arg (L858R)	COSM6224	0.5%	E1 E8 E10	3001-3002 3005-3306 3077-3078 5398-5399 5670-5671 5681-5682	NA		
	c.2573_2574TG>GT	p.Leu858Arg (L858R)	COSM12429	ND					
	c.2582T>A	p.Leu861Gln (L861Q)	COSM6213	0.3%	E9	3011-3012 3015-3016	1.3%	E1	5670-5671 5681-5682

Table 10: Sensitivity of SensiScreen® FFPE EGFR assays. Plasmid or cell-line DNA harboring the indicated EGFR mutations were serially diluted in 50 ng wild type human genomic DNA and analyzed with SensiScreen® EGFR Simplex and/or Multiplex assays using MyGo Pro (IT-IS Life Science) or Gentier 48 (Xi'AN TIANLONG SCIENCE AND TECHNOLOGY CO.) real-time PCR instruments. Limit of detection (LOD, % mutation) was calculated using the formula 9 = A ln(x) + B where A is the slope of the logarithmic trendline (R^2>98) of at least three of the serial dilution points 10%, 5%, 2%, 1% and 0.5%, and B is the value (ΔCt) at 1% mutation. *Limit of detection (LOD, % mutation) of the SensiScreen® EGFR exon 19 deletions assay based on determined LOD for the mutations p.E746_A750delELREA (COSM6223) and p.L747_A750>P (COSM12382) and in silico analysis. ^Limit of detection (LOD, % mutation) of the SensiScreen® EGFR exon 20 insertions assay based on determined LOD for the mutations p.V769_D770insASV (COSM12426), p.D770_N771insG (COSM12378) and p.H773_V774insH (COSM12377), and in silico analysis. ND: Not determined, NA: Assay not available.

9.2.3 LIMIT OF DETECTION KIT

SensiScreen® KIT FFPE Limit of detection					
Gene	**CDS mutation**	**Amino acid substitution**	**Cosmic ID**	**LOD SensiScreen® Simplex**	**LOD SensiScreen® Multiplex**
KIT D816V	c.2447A>T	Asp816Val	COSM1314	ND	NA

Table 11: Sensitivity of SensiScreen® FFPE KIT assay. ND: Not determined, NA: Assay not available.

9.2.4 LIMIT OF DETECTION KRAS

SensiScreen® KRAS FFPE Limit of detection									
Gene	CDS mutation	Amino acid substitution	Cosmic ID	SensiScreen® Simplex			SensiScreen® Multiplex		
				LOD	Assay#	Cat. No.	LOD	Assay#	Cat. No.
KRAS exon 2	p.Gly12Arg (G12R)	c.34G>C	COSM518	0.9%	K5	1721-1722 1726-1727 1920-1921 1925-1926	0.6%	K1 K2	1701-1702 1706-1707 1900-1901 1905-1906
	p.Gly12Cys (G12C)	c.34G>T	COSM516	0.3%			0.5%		
	p.Gly12Ser (G12S)	c.34G>A	COSM517	0.5%			0.8%		
	p.Gly12Val (G12V)	c.35G>T	COSM520	0.3%			0.3%		
	p.Gly12Phe (G12F)	c.34_35GG>TT	COSM512	ND			ND		
	p.Gly12Ile (G12I)	c.34_35GG>AT	COSM34144	ND			ND		
	p.Gly12Ala (G12A)	c.35G>C	COSM522	0.3%	K6	1721-1722 1731-1732 1920-1921 1930-1931	0.2%		
	p.Gly12Asp (G12D)	c.35G>A	COSM521	0.3%			0.5%		
	p.Gly13Asp (G13D)	c.38G>A	COSM532	0.4%			0.2%		
KRAS exon 3	p.Ala59Thr (A59T)	c.175G>A	COSM546	0.9%	K7	1736-1737 1935-1936 1741-1742 1940-1941	0.8%	K1 K3	1701-1702 1900-1901 1711-1712 1910-1911
	p.Gln61His (Q61H1)	c.183A>C	COSM554	0.3%			0.4%		
	p.Gln61Lys (Q61K)	c.181C>A	COSM549	0.6%			0.5%		
	p.Gln61Leu (Q61L)	c.182A>T	COSM553	0.6%			1.2%		
	p.Ala59Gly (A59G)	c.176C>G	COSM28518	0.5%	K8	1736-1737 1935-1936 1746-1747 1945-1946	1.8%		
	p.Gln61His (Q61H2)	c.183A>T	COSM555	0.7%			0.3%		
	p.Gln61Glu (Q61E)	c.181C>G	COSM550	0.5%			0.9%		
	p.Gln61Arg (Q61R)	c.182A>G	COSM552	1.5%			1.8%		
KRAS exon 4	p.Lys117Asn (K117N1)	c.351A>C	COSM19940	1.6%	K9	1751-1752 1756-1757 1950-1951 1955-1956	1.0%	K1 K4	1701-1702 1716-1717 1900-1901 1915-1916
	p.Lys117Asn (K117N2)	c.351A>T	COSM28519	1.9%			1.8%		
	p.Ala146Pro (A146P)	c.436G>C	COSM19905	0.3%	K10	1751-1752 1761-1762 1950-1951 1960-1961	1.6%		
	p.Ala146Thr (A146T)	c.436G>A	COSM19404	0.9%			1.2%		
	p.Ala146Val (A146V)	c.437C>T	COSM19900	1.1%			1.6%		

Table 12: Sensitivity of SensiScreen® FFPE KRAS assays. Plasmid or cell-line DNA harboring the indicated KRAS mutations were serially diluted in 50 ng wild type human genomic DNA and analyzed with SensiScreen® KRAS Simplex and Multiplex assays using MyGo Pro (IT-IS Life Science) real-time PCR instruments. Limit of detection (LOD, % mutation) was calculated using the formula 9 = A ln(x) + B where A is the slope of the logarithmic trendline (R^2>96) of at least three of the serial dilution points 10%, 5%, 2%, 1% and 0.5%, and B is the value (ΔCt) at 1% mutation. ND: Not determined

9.2.5 LIMIT OF DETECTION NRAS

SensiScreen® NRAS FFPE Limit of detection									
Gene	CDS mutation	Amino acid substitution	Cosmic ID	SensiScreen® Simplex			LOD SensiScreen® Multiplex		
				LOD	Assay#	Cat. No.	LOD	Assay#	Cat. No.
NRAS exon 2	p.Gly12Ala (G12A)	c.35G>C	COSM565	0.5%	N5	1791-1792 2020-2021 1796-1797 2025-2026	1.0%	N1 N2	1771-1772 1776-1777 2000-2001 2005-2006
	p.Gly12Cys (G12C)	c.34G>T	COSM562	0.6%			0.5%		
	p.Gly12Asp (G12D)	c.35G>A	COSM564	0.5%			0.8%		
	p.Gly12Arg (G12R)	c.34G>C	COSM561	1.0%			1.2%		
	p.Gly12Ser (G12S)	c.34G>A	COSM563	0.9%			2.0%		
	p.Gly12Val (G12V)	c.35G>T	COSM566	0.5%			0.5%		
	p.Gly13Ala (G13A)	c.38G>C	COSM575	0.4%	N6	1791-1792 1801-1802 2020-2021 2030-2031	0.5%		
	p.Gly13Cys (G13C)	c.37G>T	COSM570	1.2%			0.6%		
	p.Gly13Asp (G13D)	c.38G>A	COSM573	0.7%			0.4%		
	p.Gly13Arg (G13R)	c.37G>C	COSM569	0.5%			0.7%		
	p.Gly13Ser (G13S)	c.37G>A	COSM571	1.2%			1.6%		
	p.Gly13Val (G13V)	c.38G>T	COSM574	0.5%			0.7%		
NRAS exon 3	p.Gln61His (Q61H1)	c.183A>T	COSM585	0.5%	N7	1806-1807 1811-1812 2035-2036 2040-2041	0.5%	N1 N3	1771-1772 1781-1782 2000-2001 2010-2011
	p.Gln61His (Q61H2)	c.183A>C	COSM586	1.3%			1.5%		
	p.Gln61Lys (Q61K)	c.181C>A	COSM580	1.0%			1.7%		
	p.Gln61Leu (Q61L)	c.182A>T	COSM583	0.4%			0.8%		
	p.Gln61Arg (Q61R)	c.182A>G	COSM584	1.1%			1.9%		
	p.Ala59Asp (A59D)	c.176C>A	COSM253327	0.4%	N8	1806-1807 1816-1817 2035-2036 2040-2041	0.5%		
	p.Ala59Thr (A59T)	c.175G>A	COSM578	1.0%			1.2%		
NRAS exon 4	p.Lys117Asn (K117N1)	c.351G>C	NA	1.0%	N9	1821-1822 2048-2049 2050-2051 5356-5357	1.6%	N1 N4	1771-1772 1786-1787 2000-2001 2015-2016
	p.Lys117Asn (K117N2)	c.351G>T	NA	1.5%			1.4%		

	p.Ala146Pro (A146P)	c.436G>C	COSM4172577	1.9%	N10	1826-1827	1.9%	
	p.Ala146Thr (A146T)	c.436G>A	COSM27174	1.3%		2048-2049	1.7%	
	p.Ala146Val (A146V)	c.437C>T	COSM4170228	1.4%		2055-2056 5356-5357	1.2%	

Table 13: Sensitivity of SensiScreen® FFPE NRAS assays. Plasmid or cell-line DNA harboring the indicated NRAS mutations were serially diluted in 50 ng wild type human genomic DNA and analyzed with SensiScreen® NRAS Simplex and Multiplex assays using MyGo Pro (IT-IS Life Science) or Gentier 48 (XI'AN TIANLONG SCIENCE AND TECHNOLOGY CO.) real-time PCR instruments. Limit of detection (LOD, % mutation) was calculated using the formula 9 = A ln(x) + B where A is the slope of the logarithmic trendline (R^2>96) of at least three of the serial dilution points 10%, 5%, 2%, 1% and 0.5%, and B is the value (ΔCt) at 1% mutation.

9.2.6 LIMIT OF DETECTION PIK3CA

SensiScreen® PIK3CA FFPE Limit of detection					
Gene	**Amino acid substitution**	**CDS mutation**	**Cosmic ID**	**LOD SensiScreen® Simplex**	**LOD SensiScreen® Multiplex**
PIK3CA	c.3140A>T	p.H1047L	COSM776	ND	ND
	c.3140A>G	p.H1047R	COSM775	ND	ND
	c.3139C>T	p.H1047Y	COSM774	ND	ND

Table 14: Sensitivity of SensiScreen® FFPE PIK3CA assays. ND: Not determined

9.3. STRESS TOLERANCE

Freeze-thaw cycle stability

Assay performance was evaluated during repeated freeze-thaw cycles in a 24 hour and 48 hour time period. The SensiScreen® BRAF V600D assay was subjected to 8 freeze-thaw cycles. At each cycle, 6 reactions were moved into new tubes and stored at 5 degrees until analysis. The stock mix was frozen in 2 h intervals or overnight. Assay performance was found to be unchanged after 8 freeze-thaw cycles during both time periods.

In use stability

Sensitivity to light and elevated temperature (20°C) was evaluated by making a stock mixture of the SensiScreen® BRAF V600D FFPE Dispense-ready assay and distributed to a total of 20 oligo tubes – 10 brown and 10 ordinary see-through tubes respectively. The 20 tubes were exposed to light from 0 to 9 hours without receiving direct sunlight. At different time points, the tubes were one by one moved to a refrigerator (4°C and darkness). Assay performance was not affected by a 9 hour exposure to light and elevated temperature irrespective of type of tube used. The effect of exposure to direct sunlight has not been tested and should be avoided.

Stability at elevated temperature

Examination of stability after prolonged exposure to high temperature and subsequent long term storage at correct temperature (-20°C) has not been tested. Therefore, the contents of any shipment received that is no longer cold (temperature is clearly below room temperature) should be regarded as damaged and returned to PentaBase.

Long term stability

Assay performance was evaluated after storage at -20°C for 15 months. Assay performance was found to be within specifications. Current shelf life of Ready-to-use assays is however limited to 9 months and further studies are ongoing to investigate further shelf life extension.

10. CLINICAL PERFORMANCE

SensiScreen FFPE assays have been clinically validated in retrospective analyses of suitable patient cohorts.

10.1 CLINICAL PERFORMANCE OF SENSISCREEN® BRAF – COLORECTAL CANCER

FFPE samples from 100 patients with histologically confirmed colorectal cancer collected from 1996 to 2009 previously analyzed by Direct sequencing (ABIPRISM 3130 Genetic Analyzer) were retrospectively analysed for BRAF V600E mutations using Mutant-enriched PCR (ME-PCR) and SensiScreen® BRAF V600E Simplex assay. All tumours were colorectal adenocarcinomas, diagnosed at the Institute of Pathology in Locarno, Switzerland. The mutational analyses of BRAF V600E by DS, ME-PCR and SensiScreen® BRAF V600E Simplex were performed at the Institute of Pathology in Locarno (Switzerland).

	SensiScreen® V600E Simplex	Mutant-enriched PCR	Direct sequencing	Difference*
Wild type	88	88	92	0/4
V600E/E2	9	9	5	0/4
Total	97	97	97	0

Table 15: Clinical performance of SensiScreen® BRAF V600E Simplex assay. SensiScreen® BRAF V600E Simplex assay was used for retrospective analysis of FFPE samples from patients with histologically confirmed colorectal cancer and previously analyzed by Direct sequencing (ABI PRISM 3130 Genetic Analyzer) and Mutant-enriched PCR. 3 samples in the cohort were found to be invalid due to insufficient amount of DNA and were removed from the analysis. *Difference in test result between SensiScreen® BRAF V600E Simplex assay and direct sequencing or mutant-enriched PCR. Publication in preparation.

<u>SensiScreen® V600E Simplex vs. Mutant-enriched PCR:</u>

Overall agreement = 97/97 = 100%
Wilson confidence interval (confidence level 0.95) = 0.96 – 1.00

Agreement wild type = 88/88 = 100%
Wilson confidence interval (confidence level 0.95) = 0.96 – 1.00

Agreement mutation = 9/9 = 100%
Wilson confidence interval (confidence level 0.95) = 0.70 – 1.00

<u>SensiScreen® V600E Simplex vs. Direct sequencing:</u>

Overall agreement = 93/97 = 95.9%
Wilson confidence interval (confidence level 0.95) = 0.90 – 0.98

Agreement wild type = 88/92 = 95.7%
Wilson confidence interval (confidence level 0.95) = 0.89 – 0.98

Agreement mutation = 5/9 = 55.6%
Wilson confidence interval (confidence level 0.95) = 0.27 – 0.81

10.2 CLINICAL PERFORMANCE OF SENSISCREEN® BRAF - MELANOMA

FFPE tumor biopsies from 127 Danish melanoma patients previously analyzed by the Cobas 4800 BRAF V600 Mutation Test were retrospectively analyzed by SensiScreen® BRAF V600 Simplex and Multiplex. The mutational analyses of BRAF V600 by Cobas 4800 BRAF V600 Mutation Test and SensiScreen® BRAF V600 Simplex and Multiplex were performed at the Department of Pathology, Aarhus University Hospital (Denmark).

	SensiScreen® Simplex	SensiScreen® Multiplex	Cobas 4800 BRAF V600 Mutation Test	Difference*
Wild type	69	69	73 (V600 Mutation Not Detected)	4
Codon V600 Mutation	54	54	50 (V600 Mutation Detected)^	4
V600D	0	0 (Wild type)^	0 (V600 Mutation detected)^	0
V600E/E2	46	46 (V600 mutation)^	45 (V600 Mutation Detected)^	1
V600K	7	7 (V600 mutation)^	5 (V600 Mutation Detected)^	2
V600R	1	1 (V600 mutation)^	0 (V600 Mutation Detected^	1
Total	123	123	123	0

Table 16: Clinical performance of SensiScreen® BRAF Simplex and Multiplex assays. FFPE tumor biopsies from 126 Danish melanoma patients previously analyzed by the Cobas 4800 BRAF V600 Mutation Test were retrospectively analyzed by SensiScreen® BRAF Simplex and Multiplex. 4 samples in the cohort were found to be invalid and were removed from the analysis. Publication in preparation.
*Difference in test result between SensiScreen® BRAF assays and Cobas 4800 BRAF V600 Mutation Test.
^ Mutation type identified by SensiScreen® BRAF Simplex assay

SensiScreen® Simplex and Multiplex vs. Cobas 4800 BRAF V600 Mutation Test:

Overall agreement = 119/123 = 96.8%
Wilson confidence interval (confidence level 0.95) = 0.92 – 0.99

Agreement wild type = 69/73 = 94.5%
Wilson confidence interval (confidence level 0.95) = 0.87 – 0.98

Agreement mutation = 50/54 = 92.6%
Wilson confidence interval (confidence level 0.95) = 0.82 – 0.97

10.3 Clinical performance of SensiScreen® EGFR – Non-small cell lung cancer

SensiScreen® EGFR Simplex and Multiplex assays were used for retrospective analysis of genomic DNA extracted from FFPE tumor samples from patients with non-small cell lung cancer (NSCLC) previously analyzed by Direct sequencing (ABI PRISM 3130 Genetic Analyzer). The mutational analyses of EGFR by Direct Sequencing and SensiScreen® EGFR Simplex and Multiplex were performed at the Institute of Pathology in Locarno (Switzerland).

Summary:

	SensiScreen®	Direct Sequencing	Difference*
Wild type	384	398	14
G719A/C/S	4 (S+M)	4	0
Exon 19 deletions	30 (M)	26	4
Exon 20 insertions	8 (M)	6	2
T790M	8 (S)	6	2
S768I	4 (S)	4	0
L858R	24 (S)	19	5
L861Q	6 (S)	5	1
Total	468	468	0

Table 17: Clinical performance of SensiScreen® EGFR Simplex and/or Multiplex assays. FFPE tumor biopsies from 468 non-small cell lung cancer (NSCLC) patients previously analyzed by direct sequencing (ABI PRISM 3130 Genetic Analyzer) were retrospectively analyzed by SensiScreen® EGFR Simplex and/or Multiplex assays. *Difference in test result between SensiScreen® EGFR assays and Direct Sequencing. S: Simplex, M: Multiplex. Publication in preparation.

SensiScreen® Simplex and Multiplex vs. Direct Sequencing:

Overall agreement = 454/468 = 97.0%
Wilson confidence interval (confidence level 0.95) = 0.95 – 0.98

Agreement wild type = 384/398 = 96.5%
Wilson confidence interval (confidence level 0.95) = 0.94 – 0.98

Agreement mutation = 70/84 = 83.3%
Wilson confidence interval (confidence level 0.95) = 0.74 – 0.90

EGFR G719

	SensiScreen® G719A/C/S Simplex	SensiScreen® G719A/C/S Multiplex	Direct Sequencing	Difference*
Wild type	464	464	464	0
G719A	1	1 (G719A/C/S mutation)	1	0
G719C	0	0	0	0
G719S	3	3 (G719A/C/S mutation)	3	0
Total	468	468	468	0

Table 18: Clinical performance of SensiScreen® EGFR G719A/C/S Simplex and Multiplex assays. FFPE tumor biopsies from 468 non-small cell lung cancer (NSCLC) patients previously analyzed by direct sequencing (ABI PRISM 3130 Genetic Analyzer) were retrospectively analyzed by SensiScreen® EGFR G719 Simplex and Multiplex assays. *Difference in test result between SensiScreen® EGFR assays and Direct Sequencing.

EGFR Exon 19 Deletions

	SensiScreen® Exon 19 Deletions Multiplex	Direct Sequencing	Difference*
Wild type	438	442	4
E746_A750del	17 (Exon 19 Deletion)	17	0
E746_S752del	4 (Exon 19 Deletion)	4	0
L747_A750del	1 (Exon 19 Deletion)	1	0
L747_T751del	2 (Exon 19 Deletion)	2	0
L747_S753del	1 (Exon 19 Deletion)	1	0
S752_I759del	1 (Exon 19 Deletion)	1	0
Unknown Exon 19 Deletion	4 (Exon 19 Deletion)	0	4
Total	468	468	0

Table 19: Clinical performance of SensiScreen® EGFR exon 19 deletions assay. FFPE tumor biopsies from 468 non-small cell lung cancer (NSCLC) patients previously analyzed by direct sequencing (ABI PRISM 3130 Genetic Analyzer) were retrospectively analyzed by SensiScreen® EGFR exon 19 deletions assay. *Difference in test result between SensiScreen® EGFR assays and Direct Sequencing.

EGFR Exon 20 Insertions

	SensiScreen® Exon 20 Insertions Multiplex	Direct Sequencing	Difference*
Wild type	460	462	2
P772_H773 ins PR	1 (Exon 20 Insertion)	1	0
H773_V774 ins NPH	2 (Exon 20 Insertion)	2	0
D770_N771 ins G	1 (Exon 20 Insertion)	1	0
D770_N771 ins SVE	1 (Exon 20 Insertion)	1	0
K745_E746 ins IPVAIK	1 (Exon 20 Insertion)	1	0
Unknown Exon 20 Insertion	2 (Exon 20 Insertion)	0	2
Total	468	468	0

Table 20: Clinical performance of SensiScreen® EGFR exon 20 insertions assay. FFPE tumor biopsies from 468 non-small cell lung cancer (NSCLC) patients previously analyzed by direct sequencing (ABI PRISM 3130 Genetic Analyzer) were retrospectively analyzed by SensiScreen® EGFR exon 20 insertions assay.. *Difference in test result between SensiScreen® EGFR assays and Direct Sequencing.

EGFR S768I

	SensiScreen® S768I Simplex	Direct Sequencing	Difference*
Wild type	464	464	0
S768I	4	4	0
Total	468	468	0

Table 21: Clinical performance of SensiScreen® EGFR S768I assay. FFPE tumor biopsies from 468 non-small cell lung cancer (NSCLC) patients previously analyzed by direct sequencing (ABI PRISM 3130 Genetic Analyzer) were retrospectively analyzed by SensiScreen® EGFR S768I assay.
*Difference in test result between SensiScreen® EGFR assays and Direct Sequencing.

EGFR T790M

	SensiScreen® T790M	Direct Sequencing	Difference
Wild type	460	462	2
T790M	8	6	2
Total	468	468	0

Table 22: Clinical performance of SensiScreen® EGFR T790M assay. FFPE tumor biopsies from 468 non-small cell lung cancer (NSCLC) patients previously analyzed by direct sequencing (ABI PRISM 3130 Genetic Analyzer) were retrospectively analyzed by SensiScreen® EGFR T790M assay.
*Difference in test result between SensiScreen® EGFR assays and Direct Sequencing

EGFR L858R

	SensiScreen® L858R	Direct Sequencing	Difference
Wild type	444	449	5
T790M	24	19	5
Total	468	468	0

Table 23: Clinical performance of SensiScreen® EGFR L858R assay. FFPE tumor biopsies from 468 non-small cell lung cancer (NSCLC) patients previously analyzed by direct sequencing (ABI PRISM 3130 Genetic Analyzer) were retrospectively analyzed by SensiScreen® EGFR L858R assay.
*Difference in test result between SensiScreen® EGFR assays and Direct Sequencing

EGFR L861Q

	SensiScreen® L861Q Simplex	Direct Sequencing	Difference*
Wild type	462	463	1
S768I	6	5	1
Total	468	468	0

Table 24: Clinical performance of SensiScreen® EGFR L861Q assay. FFPE tumor biopsies from 468 non-small cell lung cancer (NSCLC) patients previously analyzed by direct sequencing (ABI PRISM 3130 Genetic Analyzer) were retrospectively analyzed by SensiScreen® EGFR L861Q assay.
*Difference in test result between SensiScreen® EGFR assays and Direct Sequencing

EGFR S768I+L861Q

	SensiScreen® S768I Simplex	SensiScreen® L861Q Simplex	SensiScreen® S768I+L861Q Multiplex	Ion Torrent NGS	Difference*
Wild type	13	13	11 (S768I or L861Q mutation)	12	±1
S768I	2	0	2 (S768I or L861Q mutation)	1	1
L861Q	0	2	2 (S768I or L861Q mutation)	2	0
Total	15	15	15 (S768I or L861Q mutation)	15	0

Table 25: Clinical performance of SensiScreen® EGFR S768I+L861Q Multiplex assay. The SensiScreen® EGFR S768I+L861Q Multiplex was validated on a reduced cohort of patients with non-small cell lung cancer (NSCLC) previously analyzed by SensiScreen® EGFR S786I and L861Q Simplexes and Ion Torrent sequencing. *Difference in test result between SensiScreen® EGFR assays and Ion Torrent Sequencing.

10.4 Clinical performance KIT

The clinical performance of SensiScreen® KIT assays is currently being evaluated.

10.5 Clinical performance KRAS – Colorectal cancer

SensiScreen® KRAS Exon 2, 3 and 4 Simplex and Multiplex assays were used for retrospective analysis of FFPE samples from patients with histologically confirmed colorectal cancer and previously analyzed by Direct sequencing (ABIPRISM 3130 Genetic Analyzer), Mutant-enriched PCR (Cohort 1), therascreen® KRAS Test (Cohort 2) and/or cobas® KRAS Mutation Test. Analysis of KRAS Exon 2 mutational status by Direct Sequencing and Mutant-enriched PCR was performed at the Institute of Pathology in Locarno, Switzerland while the analysis of KRAS Exon 2 mutational status by therascreen® KRAS Test and cobas® KRAS Mutation Test was performed at the Department of Pathology, Aarhus University Hospital. All tumours were colorectal adenocarcinomas, diagnosed at the Institute of Pathology in Locarno, Switzerland (Cohort 1+4) and Aarhus University Hospital, Denmark (Cohort 2+3).

Summary:

		Mutations Identified				
	Assay	**Wild type**	**KRAS Exon 2 Codon 12/13**	**KRAS Exon 3 Codon 59/61**	**KRAS Exon 4 Codon 117/146**	**Total**
Cohort 1	SensiScreen® Simplex	**45**	**44**	**6**	**4**	**99**
	SensiScreen® Multiplex	45	44	6	4	99
	Direct Sequencing	64	28	5	2	99
	Mutant-enriched PCR	56	43	NA	NA	99
	Difference	**16*/1^**	**16*/1^**	**1***	**2***	**0**
Cohort 2	SensiScreen® Simplex	60	19	NA	NA	79
	Direct Sequencing	62	17	NA	NA	79
	therascreen® KRAS Test	60	19	NA	NA	79
	Difference	**2*/0~**	**2*/0~**	**NA**	**NA**	**0**
Cohort 3	SensiScreen® Multiplex	170	93	NA	NA	283
	cobas® KRAS Mutation Test	176	87	NA	NA	283
	Difference	**6¤**	**6¤**	**NA**	**NA**	**0**
Cohort 4	SensiScreen® Simplex	54	NA	2	3	59
	SensiScreen® Multiplex	54	NA	2	3	59
	Direct Sequencing	56	NA	1	2	59
	Difference	**2***	**NA**	**1***	**1***	**0**

Table 26: Clinical performance of SensiScreen® KRAS assays. SensiScreen® KRAS Exon 2, 3 and 4 Simplex and Multiplex assays were used for retrospective analysis of FFPE samples from patients with histologically confirmed colorectal cancer and previously analyzed by Direct sequencing (ABI PRISM 3130 Genetic Analyzer, Cohort 1-2+4)), Mutant-enriched PCR (Cohort 1), therascreen® KRAS Test (Cohort 2) and/or cobas® KRAS Mutation Test (Cohort 3) Difference in test result between SensiScreen® KRAS assays and Direct Sequencing (*), Mutant-enriched PCR (^), therascreen® KRAS Test (~) and cobas® KRAS Mutation Test (¤). KRAS Exon 2 data are from Riva et al. 2017. KRAS Exon 3 and Exon 4 data are part of publication in preparation.

<u>SensiScreen® vs. Direct sequencing:</u>

Overall agreement = 214/237 = 90.3%
Wilson confidence interval (confidence level 0.95) = 0.86 – 0.93

Agreement wild type = 159/182 = 87.4%
Wilson confidence interval (confidence level 0.95) = 0.82 – 0.91

Agreement mutation = 55/78 = 70.5%
Wilson confidence interval (confidence level 0.95) = 0.60 – 0.80

SensiScreen® vs. Mutant-enriched PCR:

Overall agreement = 99/100 = 99.0%
Wilson confidence interval (confidence level 0.95) = 0.99 – 1.00

Agreement wild type = 56/57 = 98.3%
Wilson confidence interval (confidence level 0.95) = 0.91 – 1.00

Agreement mutation = 43/44 = 97.7%
Wilson confidence interval (confidence level 0.95) = 0.88 – 1.00

SensiScreen® vs. cobas® KRAS Mutation Test:

Overall agreement = 277/283 = 97.9%
Wilson confidence interval (confidence level 0.95) = 0.67 – 0.86

Agreement wild type = 60/62 = 96.6%
Wilson confidence interval (confidence level 0.95) = 0.93 – 0.98

Agreement mutation = 87/93 = 93.6%
Wilson confidence interval (confidence level 0.95) = 0.87 – 0.97

SensiScreen® vs. therascreen® KRAS test:

Overall agreement = 79/79 = 100%
Wilson confidence interval (confidence level 0.95) = 0.95 – 1.00

Agreement wild type = 60/60 = 100%
Wilson confidence interval (confidence level 0.95) = 0.94 – 1.00

Agreement mutation = 19/19 = 100%
Wilson confidence interval (confidence level 0.95) = 0.83 – 1.00

KRAS Exon 2

Cohort 1:

	SensiScreen® Simplex	SensiScreen® Multiplex	Direct Sequencing	Mutant-enriched PCR	Difference*
Wild type	55	55	71	56	16/1
KRAS Codon 12/13 Mutation	44	44	28	43	16/1
G12A	3	3 (G12A/D or G13D)	2	2	1/0
G12C	6	6 (G12R/C/S/V)	2	6	4/0
G12D	13	13 (G12A/D or G13D)	12	13	1/0
G12R	1	1	1	1	0
G12S	2	2 (G12R/C/S/V)	1	2	1/0
G12V	13	13 (G12R/C/S/V)	7	13	6/0
G13D	6	6 (G12A/D or G13D)	3	5	3/1
Total	99	99	99	99	0

Table 27: Clinical performance of SensiScreen® KRAS Exon 2 assays. SensiScreen® KRAS Exon 2 Simplex and Multiplex assays were used for retrospective analysis of FFPE samples from patients with histologically confirmed colorectal cancer and previously analyzed by Direct sequencing (ABI PRISM 3130 Genetic Analyzer) and Mutant-enriched PCR. *Difference in test result between SensiScreen® KRAS Exon 2 assays and Direct Sequencing or Mutant-enriched PCR. From Riva et al. 2017.

Cohort 2:

	SensiScreen® Simplex	Direct Sequencing	therascreen® KRAS test	Difference*
Wild type	60	62	60	2/0
KRAS Codon 12/13 Mutation	19	17	19	2/0
G12A	2	2	2	0
G12C	3	2	3	1/0
G12D	4	4	4	0
G12R	0	0	0	0
G12S	2	1	2	1/0
G12V	3	3	3	0
G13D	5	5	5	0
Total	79	79	79	

Table 28: Clinical performance of SensiScreen® KRAS Exon 2 assays. SensiScreen® KRAS Exon 2 Simplex and Multiplex assays were used for retrospective analysis of FFPE samples from patients with histologically confirmed colorectal cancer and previously analyzed by Direct sequencing (ABI PRISM 3130 Genetic Analyzer) and therascreen® KRAS Test. *Difference in test result between SensiScreen® KRAS Exon 2 assays and Direct Sequencing or therascreen® KRAS test. From Riva et al. 2017.

Cohort 3:

	SensiScreen® Multiplex	cobas® KRAS Mutation Test	Difference*
Wild type	170	176	6
KRAS Codon 12/13 Mutation	93	87	6
Total	283	283	0

Table 29: Clinical performance of SensiScreen® KRAS Exon 2 assays. SensiScreen® KRAS Exon 2 Multiplex assay was used for retrospective analysis of FFPE samples from patients with histologically confirmed colorectal cancer and previously analyzed by cobas® KRAS Mutation Test. *Difference in test result between SensiScreen® KRAS Exon 2 Multiplex assay and cobas® KRAS Mutation Test. From Riva et al. 2017.

KRAS Exon 3

Cohort 1:

	SensiScreen® Simplex	SensiScreen® Multiplex	Direct Sequencing	Difference*
Wild type	93	93	94	1
KRAS Codon 59/61 Mutation	6	6	5	1
A59G	0	0^	0	0
A59T	0	0^	0	0
Q61H1	2	2^	2	0
Q61H2	0	0^	0	0
Q61E	1	1^	0	1
Q61K	0	0^	0	0
Q61L	2	2^	2	0
Q61R	1	1^	1	0
Total	99	99	99	

Table 30: Clinical performance of SensiScreen® KRAS Exon 3 assays. SensiScreen® KRAS Exon 3 Simplex and Multiplex assays were used for retrospective analysis of FFPE samples from patients with histologically confirmed colorectal cancer and previously analyzed by Direct Sequencing. *Difference in test result between SensiScreen® KRAS assays and Direct Sequencing. ^Mutation type identified by Direct Sequencing and/or SensiScreen® KRAS Exon 3 Simplex assay. Publication in preparation.

Cohort 4:

	SensiScreen® Simplex	SensiScreen® Multiplex	Direct Sequencing	Difference*
Wild type		93	94	1
A59G	1	1^	0	0
A59T	0	0^	0	0
Q61H1	2	2^	2	0
Q61H2	0	0^	0	0
Q61E	1	1^	0	1
Q61K	0	0^	0	0
Q61L	2	2^	2	0
Q61R	1	1^	1	0
Total	59	99	99	

Table 31: Clinical performance of SensiScreen® KRAS Exon 3 assays. SensiScreen® KRAS Exon 3 Simplex and Multiplex assays were used for retrospective analysis of FFPE samples from patients with histologically confirmed colorectal cancer and previously analyzed by Direct Sequencing. *Difference in test result between SensiScreen® KRAS assays and Direct Sequencing. ^ Mutation type identified by Direct Sequencing and/or SensiScreen® KRAS Exon 3 Simplex assay. Publication in preparation.

KRAS Exon 4

Cohort 1:

	SensiScreen® Simplex	SensiScreen® Multiplex	Direct Sequencing	Difference*
Wild type	78	78	79	1
K117N1	2	2 (117N1/N2)	1	1
K117N2	0	0	0	0
A146P	0	0	0	0
A146T	1	1 (146P/T/V)	1	0
A146V	1	1 (146P/T/V)	0	1
Invalid~	15	15	18	3
Total	99	99	99	

Table 32: Clinical performance of SensiScreen® KRAS Exon 4 assays. SensiScreen® KRAS Exon 4 Simplex and Multiplex assays were used for retrospective analysis of FFPE samples from patients with histologically confirmed colorectal cancer and previously analyzed by Direct Sequencing. *Difference in test result between SensiScreen® KRAS assays and Direct Sequencing. ^Mutation type identified by Direct Sequencing and/or SensiScreen® KRAS Exon 4 Simplex assay. Publication in preparation.

Cohort 4:

	SensiScreen® Simplex	SensiScreen® Multiplex	Direct Sequencing	Difference*
Wild type	93	93	94	1
KRAS Codon 117/146 Mutation	6	6	5	1
K117N1	1	1^	0	0
K117N2	0	0	0	0
A146P	2	2^	2	0
A146T		0	0	0
A146V	1	1^	0	1
Total	99	99	99	0

Table 33: Clinical performance of SensiScreen® KRAS Exon 4 assays. SensiScreen® KRAS Exon 4 Simplex and Multiplex assays were used for retrospective analysis of FFPE samples from patients with histologically confirmed colorectal cancer and previously analyzed by Direct Sequencing. *Difference in test result between SensiScreen® KRAS assays and Direct Sequencing. ^Mutation type identified by Direct Sequencing and/or SensiScreen® KRAS Exon 4 Simplex assay. Publication in preparation.

10.6 Clinical performance NRAS – Colorectal cancer

SensiScreen® NRAS Exon 2, 3 and 4 Simplex and Multiplex assays were used for retrospective analysis of FFPE samples from patients with histologically confirmed colorectal cancer and previously analyzed by Direct sequencing (ABIPRISM 3130 Genetic Analyzer). The mutational analyses of NRAS by Direct Sequencing and SensiScreen® NRAS Exon 2, 3 and 4 Simplex and Multiplex were performed at the Institute of Pathology in Locarno (Switzerland).

Summary:

		Mutations identified				
		Wild type	NRAS Exon 2 Codon 12/13	NRAS Exon 3 Codon 59/61	NRAS Exon 4 Codon 117/146	Total
Cohort 1	**SensiScreen® Simplex**	94	2	3	NA	99
	SensiScreen® Multiplex	94	2	3	NA	99
	Direct Sequencing	96	1	2	NA	99
	Difference	3	1	2	NA	0
Cohort 4	**SensiScreen® Simplex**	54	0	5	0	59
	SensiScreen® Multiplex	54	0	5	0	59
	Direct Sequencing	57	0	2	0	59
	Difference	3	0	3	0	0
Cohort 5	**SensiScreen® Simplex**	140	NA	NA	0	140
	SensiScreen® Multiplex	140	NA	NA	0	140
	Direct Sequencing	140	0	0	0	140
	Difference	0	NA	NA	0	0

Table 34: Clinical performance of SensiScreen® NRAS assays. SensiScreen® NRAS Exon 2, 3 and 4 Simplex and Multiplex assays were used for retrospective analysis of FFPE samples from patients with histologically confirmed colorectal cancer and previously analyzed by Direct sequencing (ABI PRISM 3130 Genetic Analyzer). *Difference in test result between SensiScreen® NRAS assays and Direct Sequencing. Publication in preparation.

SensiScreen® vs. Direct Sequencing:

Overall agreement = 292/298 = 98.0%
Wilson confidence interval (confidence level 0.95) = 0.96 – 0.99

Agreement wild type = 287/293 = 98.0%
Wilson confidence interval (confidence level 0.95) = 0.96 – 0.99

Agreement mutation = 5/11 = 45.5%
Wilson confidence interval (confidence level 0.95) = 0.21 – 0.72

NRAS Exon 2

Cohort-1:

	SensiScreen® Simplex	SensiScreen® Multiplex	Direct Sequencing	Difference*
Wild type	97	97	98	1
G12A	0	0	0	0
G12C	0	0	0	0
G12D	0	0	0	0

G12R	2	2^	1	1
G12S	0	0	0	0
G12V	0	0	0	0
G13A	0	0	0	0
G13C	0	0	0	0
G13D	0	0	0	0
G13R	1	1	0	0
G13V	0	0	0	0
Total	99	99	99	0

Table 35: Clinical performance of SensiScreen® NRAS assays. SensiScreen® NRAS Exon 2 Simplex and Multiplex assays were used for retrospective analysis of FFPE samples from patients with histologically confirmed colorectal cancer and previously analyzed by Direct sequencing (ABI PRISM 3130 Genetic Analyzer). *Difference in test result between SensiScreen® NRAS assays and Direct Sequencing. ^Mutation type identified by Direct Sequencing and/or SensiScreen® NRAS Exon 2 Simplex assay. Publication in preparation.

Cohort-4:

	SensiScreen® Simplex	SensiScreen® Multiplex	Direct Sequencing	Difference*
Wild type	59	59	55	0
G12A	0	0	0	0
G12C	0	0	0	0
G12D	0	0	0	0
G12R	0	0	0	0
G12S	0	0	0	0
G12V	0	0	0	0
G13A	0	0	0	0
G13C	0	0	0	0
G13D	0	0	0	0
G13R	1	1	0	0
G13V	0	0	0	0
Invalid	0	0	4	4
Total	59	59	59	0

Table 36: Clinical performance of SensiScreen® NRAS assays. SensiScreen® NRAS Exon 2 Simplex and Multiplex assays were used for retrospective analysis of FFPE samples from patients with histologically confirmed colorectal cancer and previously analyzed by Direct sequencing (ABI PRISM 3130 Genetic Analyzer). *Difference in test result between SensiScreen® NRAS assays and Direct Sequencing. ^Mutation type identified by Direct Sequencing and/or SensiScreen® NRAS Exon 2 Simplex assay. ~Due to degradation of sample DNA. Publication in preparation.

NRAS Exon 3

Cohort-1:

	SensiScreen® Simplex	SensiScreen® Multiplex	Direct Sequencing	Difference*
Wild type	97	97	98	1
A59G	0	0^	0	0
A59T	0	0^	0	0
Q61H1	1	1^	1	0
Q61H2	0	0^	0	0
Q61E	1	1^	0	0
Q61K	1	1^	0	1
Q61L	2	2^	2	0

Q61R	1	1^	1	0
Total	99	99	99	0

Table 37: Clinical performance of SensiScreen® NRAS assays. SensiScreen® NRAS Exon 3 Simplex and Multiplex assays were used for retrospective analysis of FFPE samples from patients with histologically confirmed colorectal cancer and previously analyzed by Direct sequencing (ABI PRISM 3130 Genetic Analyzer). *Difference in test result between SensiScreen® KNAS assays and Direct Sequencing. ^Mutation type identified by Direct Sequencing and/or SensiScreen® NRAS Exon 3 Simplex assay. Publication in preparation.

Cohort-4:

	SensiScreen® Simplex	SensiScreen® Multiplex	Direct Sequencing	Difference*
Wild type	54	54	54	0
A59G	0	0^	0	0
A59T	0	0^	0	0
Q61H1	2	1^	1	1
Q61H2	0	0	0	0
Q61E	0	0	0	0
Q61K	1	1^	0	1
Q61L	0	0	0	0
Q61R	2	2^	1	1
Invalid~	0	0	3	3
Total	59	59	59	0

Table 38: Clinical performance of SensiScreen® NRAS assays. SensiScreen® NRAS Exon 3 Simplex and Multiplex assays were used for retrospective analysis of FFPE samples from patients with histologically confirmed colorectal cancer and previously analyzed by Direct sequencing (ABI PRISM 3130 Genetic Analyzer). *Difference in test result between SensiScreen® KNAS assays and Direct Sequencing. ^Mutation type identified by Direct Sequencing and/or SensiScreen® NRAS Exon 3 Simplex assay. ~Due to degradation of sample DNA. Publication in preparation.

NRAS Exon 4

Cohort-4:

	SensiScreen® Simplex	SensiScreen® Multiplex	Direct Sequencing	Difference*
Wild type	59	59	59	0
K117N1	0	0	0	0
K117N2	0	0	0	0
A146P	0	0	0	0
A146T	0	0	0	0
A146V	0	0	0	0
Total	59	59	59	0

Table 39: Clinical performance of SensiScreen® NRAS assays. SensiScreen® NRAS Exon 4 Simplex and Multiplex assays were used for retrospective analysis of FFPE samples from patients with histologically confirmed colorectal cancer and previously analyzed by Direct sequencing (ABI PRISM 3130 Genetic Analyzer). *Difference in test result between SensiScreen® KNAS assays and Direct Sequencing. ^Mutation type identified by Direct Sequencing and/or SensiScreen® NRAS Exon 4 Simplex assay. ~Due to degradation of sample DNA. Publication in preparation.

Cohort-5:

	SensiScreen® Simplex	SensiScreen® Multiplex	Direct Sequencing	Difference*
Wild type	140	140	140	0
K117N1	0	0	0	0
K117N2	0	0	0	0
A146P	0	0	0	0
A146T	0	0	0	0
A146V	0	0	0	0

Total	140	140	140	0

Table 40: Clinical performance of SensiScreen® NRAS assays. SensiScreen® NRAS Exon 4 Simplex and Multiplex assays were used for retrospective analysis of FFPE samples from patients with histologically confirmed colorectal cancer and previously analyzed by Direct sequencing (ABI PRISM 3130 Genetic Analyzer). *Difference in test result between SensiScreen® KNAS assays and Direct Sequencing. ^Mutation type identified by Direct Sequencing and/or SensiScreen® NRAS Exon 4 Simplex assay. Publication in preparation.

10.7 CLINICAL PERFORMANCE PIK3CA

The clinical performance of SensiScreen® PIK3CA assays is currently being evaluated.

11. 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 A/S 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 from 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 41: Troubleshooting

12. REFERENCES

1. **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*
2. **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.*
3. **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.*
4. **Fisher, Larkin**. Vemurafenib: a new treatment for BRAF-V600 mutated advanced melanoma. *Dove Press journal: Cancer Management and Research, August 7, 2012.*
5. **Van Cutsem et al.** ESMO consensus guidelines for the management of patients with metastatic colorectal cancer. *Annals of Oncology 0:1-38, 2016*

6. **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*.

7. **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.*

13. MANUFACTURER AND DISTRIBUTORS

11.1 MANUFACTURER

	PentaBase A/S Petersmindevej 1A 5000 Odense C, Denmark

11.2 TECHNICAL ASSISTANCE

For technical assistance in Denmark, contact PentaBase A/S:

www.pentabase.com

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.

14. SENSISCREEN® PRODUCT OVERVIEW

Gene	SensiScreen® FFPE Ready-to-use	Strip #	Catalogue # 12; 60 reactions	SensiScreen® FFPE Dispense Ready	Catalogue # 20; 50 reactions
BRAF	V600 Multiplex Ready-to-use CE IVD	B1	1831-1832	V600 Multiplex Dispense Ready CE IVD	1398-1399
	V600 Simplex Ready-to-use (V600E, V600D, V600R and V600K) CE IVD	B2	1836-1837	V600 Simplex Dispense Ready (V600E, V600D, V600R and V600K) CE IVD	1402-1403
	V600E Simplex Ready-to-use CE IVD	B3	1841-1842	V600E Simplex Dispense Ready CE IVD	1400-1401
EGFR	Exon 18+19+20+21 Multiplex Ready to use CE IVD	E1	5681-5682	Exon 18+19+20+21 Multiplex Dispense Ready CE IVD	5670-5671
	G719 Multiplex Ready-to-use CE IVD	E2	2081-2082	G719 Multiplex Dispense Ready CE IVD	2085-2086
	G719 Simplex Ready-to-us CE IVD	E3	3071-3072	G719 Simplex Dispense Ready CE IVD	3027-3028
	Del 19 Multiplex Ready-to-use CE IVD	E4	2071-2072	Del 19 Multiplex Dispense Ready CE IVD	2075-2076
	S768I Simplex Ready-to-use CE IVD	E5	2091-2092	S768I Simplex Dispense Ready CE IVD	2095-2096
	T790M Simplex Ready-to-use	E6	2061-2062	T790M Simplex Dispense Ready CE IVD	2065-2066
	Exon 20 Insertions Multiplex Ready-to-use CE IVD	E7	3021-3022	Ex20Ins Multiplex Dispense Ready CE IVD	3025-3026
	L858R Simplex Ready-to-use CE IVD	E8	3001-3002	L858R Simplex Dispense Ready CE IVD	3005-3006
	L861Q Simplex Ready-to-use CE IVD	E9	3010-3012	L861Q Simplex Dispense Ready CE IVD	3015-3016
	Del 19 Multiplex, T790M, L858R Ready-to-use CE IVD	E10	5398-5399	Del 19 Multiplex, T790M, L858R Dispense Ready CE IVD	3077-3078
KIT	D816V Simplex Ready-to-use RUO	I1	3031-3032	D816V Simplex Dispense Ready RUO	3035-3036
KRAS	Exon 2+3+4 Multiplex Ready-to-use CE IVD	K1	1701-1702	Exon 2+3+4 Multiplex Dispense Ready CE IVD	1900-1901
	Exon 2 Multiplex Ready-to-use CE IVD	K2	1706-1707	Exon 2 Multiplex Dispense Ready CE IVD	1905-1906
	Exon 3 Multiplex Ready-to-use CE IVD	K3	1711-1712	Exon 3 Multiplex Dispense Ready CE IVD	1910-1911
	Exon 4 Multiplex Ready-to-use CE IVD	K4	1716-1717	Exon 4 Multiplex Dispense Ready CE IVD	1915-1916
	Exon 2 Simplex Ready-to-use CE IVD	K5+K6	1721-1722	Exon 2 Simplex Dispense Ready CE IVD	1920-1921
	Exon 2 Simplex Ready-to-use (G12R, G12C, G12S and G12V) CE IVD	K5	1726-1727	Exon 2 Simplex A Dispense Ready (G12R, G12C, G12S and G12V) CE IVD	1925-1926
	Exon 2 Simplex Ready-to-use (G12A, G12D and G13D) CE IVD	K6	1731-1732	Exon 2 Simplex B Dispense Ready (G12A, G12D and G13D) CE IVD	1930-1931
	Exon 3 Simplex Ready-to-use CE IVD	K7+K8	1736-1737	Exon 3 Simplex Dispense Ready CE IVD	1935-1936
	Exon 3 Simplex Ready-to-use (Q61H1, Q61K, Q61L and A59T) CE IVD	K7	1741-1742	Exon 3 Simplex A Dispense Ready (Q61H1, Q61K, Q61L and A59T) CE IVD	1940-1941
	Exon 3 Simplex Ready-to-use (Q61H2, Q61E, Q61R and A59G) CE IVD	K8	1746-1747	Exon 3 Simplex B Dispense Ready (Q61H2, Q61E, Q61R and A59G) CE IVD	1945-1946
	Exon 4 Simplex Ready-to-use CE IVD	K9+K10	1751-1752	Exon 4 Simplex Dispense Ready CE IVD	1950-1951
	Exon 4 Simplex Ready-to-use (K117N1 and K117N2) CE IVD	K9	1756-1757	Exon 4 Simplex Dispense Ready (K117N1 and K117N2) CE IVD	1955-1956
	Exon 4 Simplex Ready-to-use (A146P, A146T and A146V) CE IVD	K10	1761-1762	Exon 4 Simplex Dispense Ready (A146P, A146T and A146V) CE IVD	1960-1961
NRAS	Exon 2+3+4 Multiplex Ready-to-use CE IVD	N1	1771-1772	Exon 2+3+4 Dispense Ready CE IVD	2000-2001
	Exon 2 Multiplex Ready-to-us CE IVD	N2	1776-1777	Exon 2 Multiplex Dispense Ready CE IVD	2005-2006
	Exon 3 Multiplex Ready-to-use CE IVD	N3	1781-1782	Exon 3 Multiplex Dispense Ready CE IVD	2010-2011
	Exon 4 Multiplex Ready-to-use CE IVD	N4	1786-1787	Exon 4 Multiplex Dispense Ready CE IVD	2015-2016
	Exon 2 Simplex Ready-to-use CE IVD	N5+N6	1791-1792	Exon 2 Simplex Dispense Ready CE IVD	2020-2021
	Exon 2 Simplex Ready-to-use (G12A, G12C, G12D, G12R, G12S and G12V) CE IVD	N5	1796-1797	Exon 2 Simplex Dispense Ready (G12A, G12C, G12D, G12R, G12S and G12V) CE IVD	2025-2026
	Exon 2 Simplex Ready-to-use (G13A, G13C, G13D, G13R, G13S and G13V) CE IVD	N6	1801-1802	Exon 2 Simplex Dispense Ready (G13A, G13C, G13D, G13R, G13S and G13V) CE IVD	2030-2031
	Exon 3 Simplex Ready-to-use CE IVD	N7+N8	1806-1807	Exon 3 Simplex Dispense Ready CE IVD	2035-2036
	Exon 3 Simplex Ready-to-use (Q61H1, Q61H2, Q61K, Q61L and Q61R) CE IVD	N7	1811-1812	Exon 3 Simplex A Dispense Ready (Q61H1, Q61H2, Q61K, Q61L and Q61R) CE IVD	2040-2041
	Exon 3 Simplex Ready-to-use (A59D and A59T) CE IVD	N8	1816-1817	Exon 3 Simplex B Dispense Ready (A59D and A59T) CE IVD	2045-2046
	Exon 4 Simplex Ready-to-use CE IVD	N9+N10	5356-5357	Exon 4 Simplex Dispense Ready CE IVD	2048-2049
	Exon 4 Simplex Ready-to-use (N117N1 and N117N2) CE IVD	N9	1821-1822	Exon 4 Simplex Dispense Ready (N117N1 and N117N2) CE IVD	2050-2051
	Exon 4 Simplex Ready-to-use (A146P, A146T and A146V) CE IVD	N10	1826-1827	Exon 4 Simplex Dispense Ready (A146P, A146T and A146V) CE IVD	2055-2056
PIK3CA*	PIK3CA Multiplex Ready-to-use RUO	P1	3041-3042	PIK3CA Multiplex Dispense Ready CE IVD	3045-3046
	PIK3CA Simplex Ready-to-use (H1047R, H1047Y and H1047L) RUO	P2	3051-3052	PIK3CA Simplex Dispense Ready (H1047R, H1047Y and H1047L) CE IVD	3055-3056

Table 42: Product overview

15. 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 Applera Corporation or its subsidiaries in the U.S. and certain other countries. Inc. SensiScreen®, HydrolEasy™, SuPrimers™ and BaseBlockers™ are all trademarks belonging to PentaBase A/S. Products or parts of it must not be resold or transferred without PentaBases acceptance. PentaBase A/S takes certain reservation for changes. PentaBase A/S disclaim all responsibility for any errors that may appear in this Instructions for use. Furthermore, PentaBase A/S 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.

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15.1 DATE OF REVISION

SensiScreen® protocol was revised September 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. Three EGFR combination products (product codes) has been added to the table on page 20	3.12
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).	3.16

		Changed the nomenclature of the references in table 2+3 Added guidelines about baseline correction and internal control analysis (section 8)	
3.22	January 2018	Changed the Cat. no. of EGFR Strip E10	3.21
3.23	February 2018	Added the Mic Real Time PCR cycler to validated real time PCR 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
3.4	February 2019	Updated list of mutations detected (table 1) and product overview table	3.3
3.5	August 2019	Added Non-clinical and clinical performance data (sections 9 and 10). Updated list of validated real-time PCR instruments (section 4.5), shipment information (section 5.2) and recommended procedure for extraction of genomic DNA (section 6.1).	3.4
3.6	September 2019	Corrected sensitivity data for EGFR, KRAS and NRAS (table 10 + 12-13). Added clinical data for BRAF V600E in colorectal cancer (table 15). Removed invalid samples from statistical analysis of BRAF V600 in Melanoma (Table 16).	3.5
3.7	September 2019	Added information about design and execution of clinical evaluation (Section 10).	3.6

Table 43: Change history

16. PUBLICATIONS

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.*

17. NOTES