INSTRUCTIONS FOR USE
SENSISCREEN® LIQUID ASSAYS

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

Version: 1.9.1

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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 1 copy of human DNA in liquid biopsies.

SensiScreen® liquid assays should be used on human DNA samples from liquid biopsies or fresh frozen material by trained laboratory personnel in a professional laboratory environment. 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 a "Quick guide". The "Instructions for use" will be 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 using real-time PCR analysis. SensiScreen® is based on PentaBase's highly sensitive DNA technology that makes it possible to detect down to a single copy of mutated DNA in a background of wild type DNA. 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	c.2240_2251del12	p.L747_T751>S	COSM6210	
	c.2239_2247del9	p.L747_E749delLRE	COSM6218	
	c.2238_2255del18	p.E746_S752>D	COSM6220	
	c.2235_2249del15	p.E746_A750delELREA	COSM6223	
	c.2236_2250del15	p.E746_A750delELREA	COSM6225	
	c.2235_2246del12	p.E746_E749delELRE	COSM28517	
	c.2239_2256del18	p.L747_S752delLREATS	COSM6255	
	c.2237_2254del18	p.E746_S752>A	COSM12367	
	c.2240_2254del15	p.L747_T751delLREAT	COSM12369	
	c.2240_2257del18	p.L747_P753>S	COSM12370	
	c.2239_2248>C (complex)	p.L747_A750>P	COSM12382	
	c.2239_2251>C (complex)	p.L747_T751>P	COSM12383	
	c.2237_2255>T (complex)	p.E746_S752>V	COSM12384	
	c.2235_2255>AAT (complex)	p.E746_S752>I	COSM12385	
	c.2237_2252>T (complex)	p.E746_T751>V	COSM12386	
	c.2239_2258>CA (complex)	p.L747_P753>Q	COSM12387	
	c.2239_2256>CAA (complex)	p.L747_S752>Q	COSM12403	
	c.2237_2253>TTGCT (complex)	p.E746_T751>VA	COSM12416	
	c.2238_2252>GCA (complex)	p.L747_T751>Q	COSM12419	
	c.2238_2248>GC (complex)	p.L747_A750>P	COSM12422	
	c.2237_2251del15	p.E746_T751>A	COSM12678	
	c.2236_2253del18	p.E746_T751delELREAT	COSM12728	
	c.2235_2248>AATTC (complex)	p.E746_A750>IP	COSM13550	
	c.2235_2252>AAT (complex)	p.E746_T751>I	COSM13551	
	c.2235_2251>AATTC (complex)	p.E746_T751>IP	COSM13552	
	c.2237_2257>TCT (complex)	p.E746_P753>VS	COSM18427	
	c.2237_2251del15	p.L747_T751delLREAT	COSM23571	
	c.2233_2247del15	p.K745_E749delKELRE	COSM26038	
	c.2234_2248del15	p.K745_A750>T	COSM1190791	
	c.2236_2248>CAAC (complex)	p.E746_A750>QP	COSM13557	
	c.2232_2249del18	p.K745_A750delKELREA	COSM221565	
	c.2237_2253>TA (complex)	p.E746_T751>V	COSM133192	
	c.2239_2257>T (complex)	p.L747_P753>S	COSM133197	
	c.2239_2253>AAT (complex)	p.L747_T751>N	COSM51503	
	c.2236_2259>ATCTCG (complex)	p.E746_P753>IS	COSM133191	
EGFR exon 20	c.2369C>T	p.Thr790Met (T790M)	COSM6240	
	c.2303G>T	p.Ser768Ile	COSM6241	
	c.2300_2301insCAGCGTGGA	p.D770_N771insSVD	COSM3728433	Multiplex 1
	c.2302_2303insCGCTGGCCA	p.A767_S768insTLA	COSM12425	Multiplex 1
	c.2307_2308ins15	p.V769_D770insMASVD	COSM28638	Multiplex 1
	c.2307_2308insGCCAGCGTG	p.V769_D770insASV	COSM12376	Multiplex 1
	c.2308_2309insCCAGCGTGG	p.V769_D770insASV	COSM12426	Multiplex 1
	c.2308_2309insGGGTCGTGG	p.V769_D770insGVV	COSM18430	Multiplex 1
	c.2308_2309insGTT	p.D770>GY	COSM12427	Multiplex 1
	c.2309_2310AC>CCAGCGTGGAT	p.V769_D770insASV	COSM13558	Multiplex 1
	c.2310_2311insAGCGTGGAC	p.D770_N771insSVD	COSM85749	Multiplex 1
	c.2310_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 investigated mutations

3. TECHNOLOGY AND REAGENTS

SensiScreen® assays combine allele-specific PCR 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 of the oligonucleotides. 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 and selectively (down to one copy of mutation in a wild type background of up to 1600 copies (0-5 ng) of DNA input per well/vial), by real-time PCR analysis.

3.1 HYDROLEASY™ PROBES

A **HydrolEasy™** probe is like a standard hydrolysis probe (also referred to as a TaqMan® probe) labeled with a fluorophore at the 5' end, and 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_{Ex}. 495 nm and λ_{Em}. 516 nm, detected on the same channel as FAM™) in combination with a Green Quencher™, and internal controls are labeled with PentaYellow™ (λ_{Ex}. 533 nm and λ_{Em}. 557 nm, detected on the same channel as HEX™, VIC®, TET™) in combination with a 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 DNA targets, suppressing false positive signals from the wild type template 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 in Figure 1.

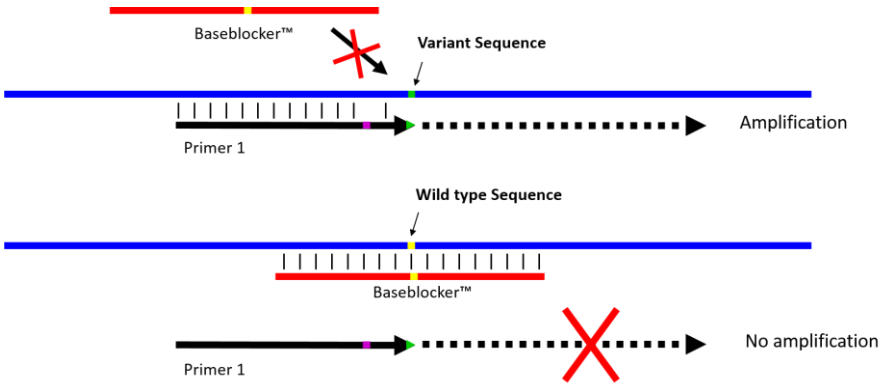

Figure 1: Illustration of how BaseBlockers™ function in SensiScreen® assays. A BaseBlocker™ binds to and blocks the wild type template from being amplified. In contrast, the BaseBlocker™ does not inhibit amplification of a template with a single nucleotide mutation and the result is a selective amplification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

4. ASSAY FORMAT

4.1 FORMAT

SensiScreen® Liquid 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)

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 table 1.	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: SensiScreen® LIQUID Ready-to-use assays. Each tube contains 20 µL in total (7.5 µL primer/probe-mix and 12.5 µL 2X AmpliQueen™ master mix). *Research use only.

BRAF V600 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 5-6	NRAS exon 4 A146V Simplex Mastermix	A146V	NRAS Reference 5	150 µL 1000 µL	375 µL 2500 µL
PIK3CA H1047 Assays						
Gene	**Tube #**	**Content**	**Mutations**	**Corresponding reference**	**Volume 20x**	**Volume 50x**
PIK3CA Multiplex*	1 2 3	**PIK3CA Reference 1** PIK3CA Multiplex Mastermix	H1047L, H1047R, H1047Y	PIK3CA Reference 1	150 µL 150 µL 300 µL	375 µL 375 µL 1250 µL
PIK3CA Simplex*	1 2 3 4 5-6	**PIK3CA Reference 1** PIK3CA H1047L Simplex PIK3CA H1047R Simplex PIK3CA H1047Y Simplex Mastermix	 H1047L H1047R H1047Y	 PIK3CA Reference 1 PIK3CA Reference 1 PIK3CA Reference 1	150 µL 150 µL 150 µL 150 µL 1000 µL	375 µL 375 µL 375 µL 375 µL 2500 µL

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

4.2 Internal control

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 DNA 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.

4.4 Mutation assay

The mutation assays (see Table 2 and 3) are used for the detection of the specified mutation(s) in a sample, and a valid signal reveals the presence of the mutation(s) (see section 8 “Data Analysis”). SensiScreen® mutation assays all contain a HydrolEasy™ probe labeled 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 Ct values of the mutations assays(s) and the corresponding reference are 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 (eg. extracted cell-free DNA (cfDNA) from liquid biopsies)
- Real-Time PCR instrument*
- Plasticware (tubes/plates) that is 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

* SensiScreen® Liquid has been validated on the following real-time PCR instruments: MyGo Pro and MyGo Mini (IT-IS Life Science Ltd.); Rotorgene (Qiagen); CFX96 (BioRad); Mx3000P and Mx3005P (Stratagene); PikoReal (Thermo Fisher); ABI 7500 and PRISM®7900HT (Applied Biosystems), Mic (bio molecular systems) and Lightcycler®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 assays, 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 use
- 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 DNA addition. Dedicated pipettes should be used and it is recommended to have separate areas for sample preparation and PCR running.
- PCR tubes should not be opened after completing the PCR program
- All used instruments and equipment should be calibrated and meet their original specifications

5.2 Shipment

SensiScreen® Ready-to-use assays are shipped on dry ice while Dispense Ready assays are shipped on either dry ice or blue ice. If the SensiScreen® packaging has been opened during transport or if the products are not frozen upon arrival, please contact your local distributor or PentaBase A/S (see section 13 “Additional Information”). 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 on the label of the box.

6. Specimens

Specimens should be human DNA (eg. cfDNA) extracted from liquid biopsies or fresh frozen samples. The samples should be collected and stored after standard pathology methodologies to ensure optimal quality. When analyzing cfDNA, it is recommended to process the biopsy right after sampling due to the short half-life of cfDNA (down to 15 min.). Extracted DNA should be stored at maximum -18˚C until use.

6.1 Recommended procedure for extraction of cfDNA from liquid biopsies

Several methods to extract cfDNA from liquid biopsies can be used. Various methods have been validated under the development and validation of SensiScreen®, among other Maxwell® RSC ccfDNA Plasma Kit (Promega, Cat. #AS1480). Regardless of method, it is recommended to use bead-based methods and follow the manufacturers protocol for cfDNA 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 reduce freeze/thaw cycles. For each sample, a reference assay must be included in the mutation analysis (See Table 2 and 3). Reference assays 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 DNA (0-1 ng/µL) to the mutation assays and the corresponding reference in this order, and mix carefully by pipetting. It is recommended to include a no template control (NTC) in each run. Add nuclease-free H_2O instead of DNA
- Close lids and spin down. Check for air bubbles and spin or tap tubes to remove bubbles from well bottoms and sides.
- 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 DNA (0-1 ng/µL) from each sample to the mutation assays and the corresponding reference in this order, and mix carefully by pipetting. It is recommended to include a NTC in each run. Add nuclease-free H_2O to the NTC instead of DNA
- Seal all tubes/wells and spin down. Make sure that there are no bubbles 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 guide lines. For more information, see section 8 "Data analysis"

Example of 96 well layout for KRAS exon 2 Dispense Ready												
	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 of KRAS exon 2 Liquid simplex assay

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

A sample is positive for a given mutation if the reference assay Ct is acceptable (Table 7) and the Ct of the mutation assay is at or lower than 40. **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 Ct values are within the specified range for each mutation.

8.3 Analysis protocol

Use the following protocol to determine the mutational status:

1. Analyse the mutation assay against the corresponding reference for one sample at a time
2. Correct for "baseline drift" before setting the threshold:
    i. Use slope correction/curve fitting when possible and/or define the baseline or background cycle interval to be between cycle 16 and cycle 26
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)

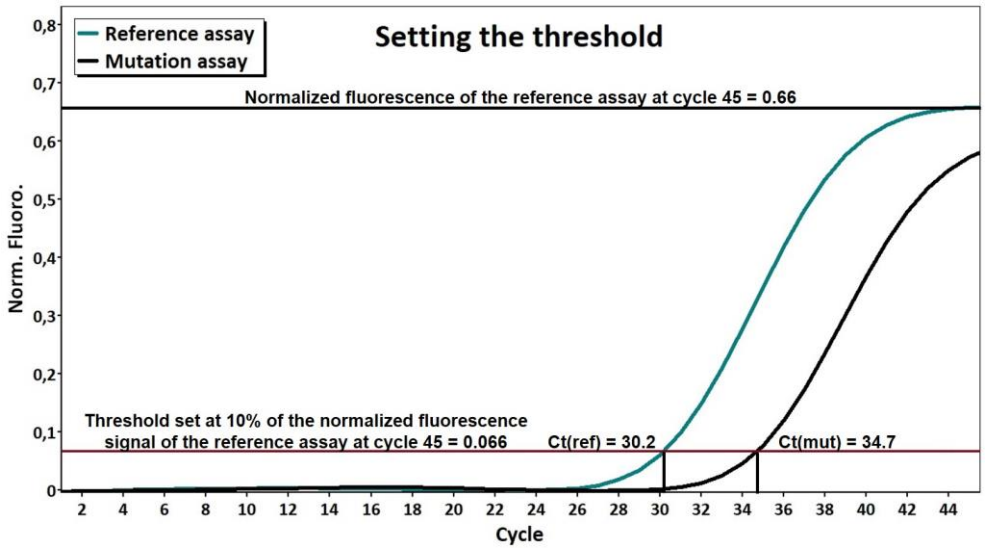

Figure 2: Setting the threshold. Read the fluorescence value for the reference assay at cycle 45 and set the threshold at 10% of this value. This setting for the threshold is now used for the analysis of the corresponding mutation assay(s). In the shown example, Ct(reference)=30.2 and Ct(mutation)=34.7.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

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

Ct for reference	Quality	Comments
Ct, reference <29	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 or using the SensiScreen® FFPE version
29≤ Ct, reference ≤40	Acceptable*	The amount of input DNA is acceptable for the mutation analysis. ***Please note that the closer the reference Ct is to 29, the higher the number of DNA templates analysed and thus the higher the probability is of detecting mutated DNA diluted in a wild type background.**
Ct, reference >40	Not valid	The amount of amplifiable 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.

5. Read the Ct value for the corresponding reference assay and validate that the reference sample is suitable for analysis cf. Table 7.

Ct for assay	Conclusion	Comments
Ct ≤40	Positive	The sample is positive for the mutation if Ct ≤40 for the mutation assay
Ct >40	Negative	The sample is negative for the mutation if Ct >40 for the mutation assay

Table 8: Mutation analysis.

6. Read the Ct value for the mutation assay(s). The sample is positive for the mutation(s) of interest if the Ct of the mutation assay(s) is equal to or lower than 40. The sample is negative for the mutation(s) of interest if the Ct of the mutation assay is above 40 cf. Table 8.

Optional: For quantification of the mutation load in a sample, the ΔCt value can be calculated for each mutation-specific analysis and is calculated as the difference between the Ct value from the given mutation assay subtracted the Ct value from the corresponding reference assay. ΔCt can be calculated for all mutation assays having a Ct value lower than 40. The theoretical mutant allele frequency based on the calculated ΔCt value is described in Table 9.

$$\Delta Ct = Ct_{mutation} - Ct_{reference}$$

ΔCt	Mutant allele frequency	Ct	Copies (using 5 ng DNA)
ΔCt 3	≈ 10 %	≈ 32	≈ 160
ΔCt 7	≈ 1 %	≈ 35	≈ 16
ΔCt 8	≈ 0.5 %	≈ 36	≈ 8
ΔCt 8.5	≈ 0.3 %	≈ 37	≈ 5
ΔCt 9	≈ 0.2 %	≈ 38	≈ 3
ΔCt 10	≈ 0.1 %	≈ 39	1-2

Table 9: Theoretical mutant allele frequency and absolute copy number in 5 ng sample approximated.

8.4 Internal control analysis

In reactions with no or late amplification by the PentaGreen™ labeled assay, it should be validated that template has been added and/or amplification has taken place by examining the fluorescence from the internal control assay (yellow channel). To set the threshold for the internal control assay, select the yellow channel and repeat steps 1-3 in section 8.3. **Note:** The internal control assay contains suboptimal primer concentrations and amplification may be inhibited by amplification by the PentaGreen™ labeled assay in the same reaction. Thus, the Ct value of the internal control assay is only indicative of the amount of template added to the reaction and cannot be used for precise quantification of DNA.

9. TROUBLESHOOTING

The troubleshooting guide shown in Table 10 below covers some of the most frequent questions and problems that can occur with the use of SensiScreen® and how they might be solved.

Problem	Solution
NTC signal	The assay is contaminated. Find the cause of contamination by checking all sources such as water, pipettes or facilities. If the contamination can't be located, contact Pentabase 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 assay 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.
$Ct_{reference}$< 29	The amount of input DNA is too high. This can affect the performance of SensiScreen® and make false positive signals. Repeat the PCR with lower input of DNA if possible
$Ct_{reference}$ > 40	The amount of input DNA is too low. If possible, repeat the PCR with higher input of DNA. If the mutation-specific analysis is positive, the sample is most likely mutated

Table 10. Troubleshooting.

10. REFERENCES

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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.*

11. MANUFACTURER AND DISTRIBUTORS

11.1 MANUFACTURER

PentaBase A/S

Petersmindevej 1A 19G

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.

12. SENSISCREEN® LIQUID PRODUCT OVERVIEW

Gene	SensiScreen® Liquid Ready-to-use	Strip #	Catalogue # 12; 60 reactions	SensiScreen® Liquid Dispense Ready	Catalogue # 20; 50 reactions
BRAF	V600 Multiplex Ready-to-us RUO	B1	5336-5337	V600 Multiplex Dispense Ready RUO	5525-5526
	V600 Simplex Ready-to-use (V600E, V600D, V600R and V600K) CE IVD	B2	5341-5342	V600 Simplex Dispense Ready (V600E, V600D, V600R and V600K) CE IVD	5530-5531
	V600E Simplex Ready-to-use CE IVD	B3	5346-5347	V600E Simplex Dispense Ready CE IVD	5535-5536
EGFR	Exon 18+19+20+21 Multiplex Ready to use RUO	E1	5686-5687	Exon 18+19+20+21 Multiplex Dispense Ready RUO	5675-5676
	G719 Multiplex Ready-to-use RUO	E2	5366-5367	G719 Multiplex Dispense Ready RUO	5550-5551
	G719 Simplex Ready-to-use RUO	E3	5391-5392	G719 Simplex Dispense Ready RUO	5575-5576
	Del 19 Multiplex Ready-to-use CE IVD	E4	5361-5362	Del 19 Multiplex Dispense Ready CE IVD	5545-5546
	S768I Simplex Ready-to-use RUO	E5	5371-5372	S768I Simplex Dispense Ready RUO	5555-5556
	T790M Simplex Ready-to-use CE IVD	E6	5351-5352	T790M Simplex Dispense Ready CE IVD	5540-5541
	Exon 20 Insertions Multiplex Ready-to-use RUO	E7	5386-5387	Ex20Ins Multiplex Dispense Ready RUO	5570-5571
	L858R Simplex Ready-to-use CE IVD	E8	5376-5377	L858R Simplex Dispense Ready CE IVD	5560-5561
	L861Q Simplex Ready-to-use RUO	E9	5381-5382	L861Q Simplex Dispense Ready RUO	5565-5566
	Del 19 Multiplex; T790M; L858R Ready-to-use CE IVD	E10	5408-5409	Del 19 Multiplex; T790M; L858R Dispense Ready CE IVD	3075-3076
KIT	KIT D816V Simplex Ready-to-use RUO	I1	5581-5582	KIT D816V Simplex Dispense Ready RUO	5800-5801
KRAS	Exon 2+3+4 Multiplex Ready-to-use RUO	K1	5201-5202	Exon 2+3+4 Multiplex Dispense Ready RUO	5395-5396
	Exon 2 Multiplex Ready-to-use RUO	K2	5206-5207	Exon 2 Multiplex Dispense Ready RUO	5400-5401
	Exon 3 Multiplex Ready-to-use RUO	K3	5211-5212	Exon 3 Multiplex Dispense Ready RUO	5405-5406
	Exon 4 Multiplex Ready-to-use RUO	K4	5216-5217	Exon 4 Multiplex Dispense R½eady RUO	5410-5411
	Exon 2 Simplex Ready-to-use RUO	K5+K6	5221-5222	Exon 2 Simplex Dispense Ready RUO	5415-5416
	Exon 2 Simplex Ready-to-use (G12R, G12C, G12S and G12V) RUO	K5	5226-5227	Exon 2 Simplex A Dispense Ready (G12R, G12C, G12S and G12V) RUO	5420-5421
	Exon 2 Simplex Ready-to-use (G12A, G12D and G13D) RUO	K6	5231-5232	Exon 2 Simplex B Dispense Ready (G12A, G12D and G13D) RUO	5425-5426
	Exon 3 Simplex Ready-to-use RUO	K7+K8	5236-5237	Exon 3 Simplex Dispense Ready RUO	5430-5431
	Exon 3 Simplex Ready-to-use (Q61H1, Q61K, Q61L and A59T) RUO	K7	5241-5242	Exon 3 Simplex A Dispense Ready (Q61H1, Q61K, Q61L and A59T) RUO	5435-5436
	Exon 3 Simplex Ready-to-use (Q61H2, Q61E, Q61R and A59G) RUO	K8	5246-5247	Exon 3 Simplex B Dispense Ready (Q61H2, Q61E, Q61R and A59G) RUO	5440-5441
	Exon 4 Simplex Ready-to-use RUO	K9+K10	5251-5252	Exon 4 Simplex Dispense Ready RUO	5445-5446
	Exon 4 Simplex Ready-to-use (K117N1 and K117N2) RUO	K9	5256-5257	Exon 4 Simplex Dispense Ready (K117N1 and K117N2) RUO	5450-5451
	Exon 4 Simplex Ready-to-use (A146P, A146T and A146V) RUO	K10	5261-5262	Exon 4 Simplex Dispense Ready (A146P, A146T and A146V) RUO	5455-5456
NRAS	Exon 2+3+4 Multiplex Ready-to-use RUO	N1	5271-5272	Exon 2+3+4 Dispense Ready RUO	5460-5461
	Exon 2 Multiplex Ready-to-us RUO	N2	5276-5277	Exon 2 Multiplex Dispense Ready RUO	5465-5466
	Exon 3 Multiplex Ready-to-use RUO	N3	5281-5282	Exon 3 Multiplex Dispense Ready RUO	5470-5471

	Exon 4 Multiplex Ready-to-use RUO	N4	5286-5287	Exon 4 Multiplex Dispense Ready RUO	5475-5476
	Exon 2 Simplex Ready-to-use RUO	N5+N6	5291-5292	Exon 2 Simplex Dispense Ready RUO	5480-5481
	Exon 2 Simplex Ready-to-use (G12A, G12C, G12D, G12R, G12S and G12V) RUO	N5	5296-5297	Exon 2 Simplex Dispense Ready (G12A, G12C, G12D, G12R, G12S and G12V) RUO	5485-5486
	Exon 2 Simplex Ready-to-use (G13A, G13C, G13D, G13R, G13S and G13V) RUO	N6	5301-5302	Exon 2 Simplex Dispense Ready (G13A, G13C, G13D, G13R, G13S and G13V) RUO	5490-5491
	Exon 3 Simplex Ready-to-use RUO	N7+N8	5306-5307	Exon 3 Simplex Dispense Ready RUO	5495-5495
	Exon 3 Simplex Ready-to-use (Q61H1, Q61H2, Q61K, Q61L and Q61R) RUO	N7	5311-5312	Exon 3 Simplex A Dispense Ready (Q61H1, Q61H2, Q61K, Q61L and Q61R) RUO	5500-5501
	Exon 3 Simplex Ready-to-use (A59D and A59T) RUO	N8	5316-5317	Exon 3 Simplex B Dispense Ready (A59D and A59T) RUO	5505-5506
	Exon 4 Simplex Ready-to-use RUO	N9+N10	5321-5322	Exon 4 Simplex Dispense Ready RUO	5510-5511
	Exon 4 Simplex Ready-to-use (N117N1 and N117N2) RUO	N9	5326-5327	Exon 4 Simplex Dispense Ready (N117N1 and N117N2) RUO	5515-5516
	Exon 4 Simplex Ready-to-use (A146P, A146T and A146V) RUO	N10	5331-5332	Exon 4 Simplex Dispense Ready (A146P, A146T and A146V) RUO	5520-5521
PIK3CA	PIK3CA Multiplex Ready-to-use RUO	P1	5586-5587	PIK3CA Multiplex Dispense Ready RUO	5810-5811
	PIK3CA Simplex Ready-to-use (H1047R, H1047Y and H1047L) RUO	P2	5591-5592	PIK3CA Simplex Dispense Ready (H1047R, H1047Y and H1047L) RUO	5805-5806

13. ADDITIONAL INFORMATION

The CE IVD labeled SensiScreen® assays is medical equipment intended for *in vitro* diagnostic in compliance with EU's 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 PentaBase's 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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13.1 DATE OF REVISION

SensiScreen® protocol was revised January 2021

Changes from previous version

PROCEDURE No.	Effective Date	Significant Changes	Previous PROCEDURE No.
1.1	27.09.2017	Added complete EGFR assay (E1) and renamed remaining	1

		EGFR assays.	
1.2	06.12.2017	Changed configuration of EGFR Multiplex (E1) assay. Added KIT (I1) Myd88 (Y1) to product overview.	1.1
1.3	19.12.2017	Added EGFR Exon 19 Deletions; T790M; L858R assay (strip E10)	1.2
1.31	05.01.2018	Updated EGFR Exon 19 Deletions; T790M; L858R (strip E10) information	1.3
1.41	January 2018	Revised sections 1, 2, 3.2, 4.2-4.5, 5, 7.2, 8, 10 and 11 including figures (1+2) and tables (2-3, 6-8). Changed the nomenclature of the references in table 2+3 Added guidelines about baseline correction and internal control analysis (section 8) Changed Cat. No. of EGFR strip E10	1.31
1.42	February 2018	Added the Mic Real Time PCR Cycler to validated real time PCR instruments	1.41
1.43	June 2018	Removed MYD88 from the list. MYD88 is from this date a PlentPlex™ assay	1.42
1.5	August 2018	Changed the acceptable Ct cutoff from 39 to 40 for all instruments Changed the baseline cycle interval from 15-20 to 16-26	1.43
1.6	February 2019	Updated product overview table	1.5
1.7	February 2019	Updated list of mutations detected (table 1) and product overview table	1.6
1.8	August 2019	Added NRAS A59D and A59T mutations to table 1	1.7
1.9	January 2021	Layout changes	1.8

14. NOTES