Data Sheet

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Trusted Answers

SureSelect Cancer Tumor-Specific Assays

Tumor genomic profiling for targeted insights

Key Advantages

- Small panels of approximately 50 genes each for lung, colon, pancreas, bladder, and kidney
- Lower cost tumor genomic profiling
- Globally curated, clinically relevant biomarker content
- Fast turnaround time to sequencing results
- Enzymatic fragmentation option eliminates the need for physical shearing equipment

Introduction

Agilent SureSelect Cancer Tumor-Specific assays offer genomic profiling of solid tumors with next-generation sequencing (NGS) panels for lung, colon, pancreas, bladder, and kidney. These tumor-specific assays are subsets of content from the Agilent SureSelect Cancer CGP assay, which is comprised of globally curated genes sourced from cancer databases and leading clinical cancer researchers. Each assay enables detection of key classes of somatic changes, including single nucleotide variants (SNVs), copy number variants (CNVs), insertions/deletions (indels), and translocations (TLs). For gene fusion detection, these tumor-specific panels can also be paired with the SureSelect Cancer CGP RNA assay (80 genes) and multiplexed in the same sequencing run. The panels are comprised of DNA modules of approximately 50 genes each for sequencing on Illumina MiSeq and MiniSeq instruments, enabling tumor genomic profiling at a lower cost.

Like the SureSelect Cancer CGP assay, the tumor-specific assays were developed using the streamlined and high-performance Agilent SureSelect XT HS2 library preparation and target enrichment chemistry. The assays feature a fast, 90-minute hybridization step, compatibility with input as low as 10 ng, and a single-day workflow to generate sequencing-ready libraries. For optimal convenience, the assays also include enzymatic fragmentation, eliminating the need for physical shearing equipment. Maximize workflow efficiency and minimize time at the bench using the Agilent Magnis NGS Prep system, a fully automated, walkaway platform that only requires 15 minutes of hands-on time for generating sequencing-ready libraries.

Flexible Workflow to Fit Your Lab

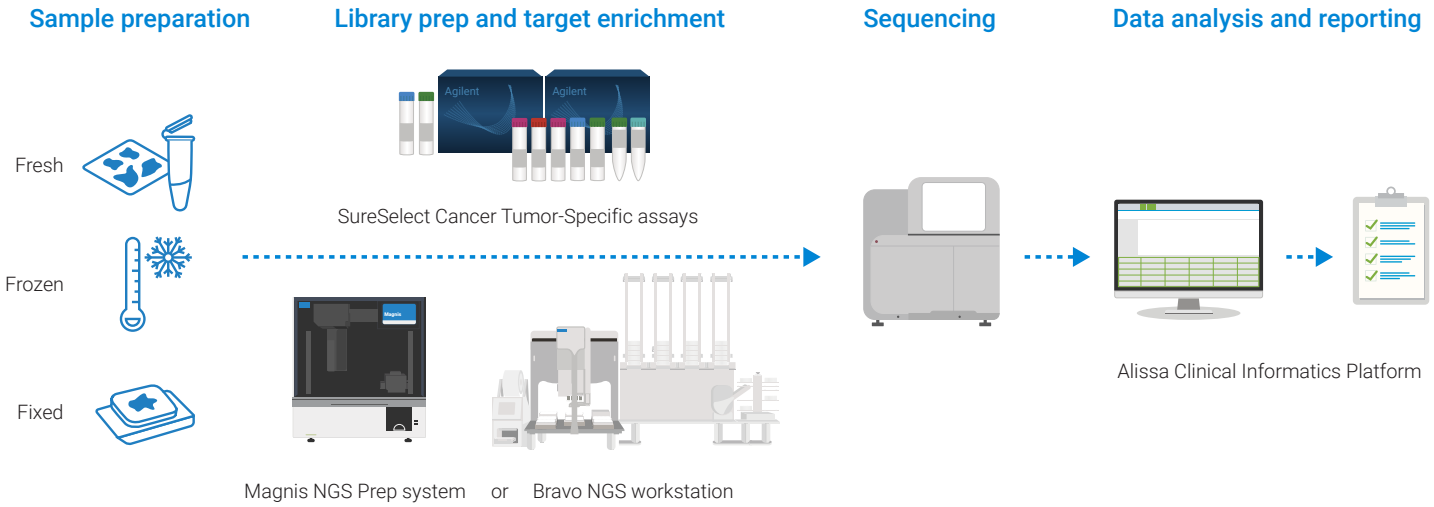

Figure 1. The Agilent SureSelect Cancer Tumor-Specific assay workflow enables laboratory scientists to take tissue specimens to sequencing results in less than four days. The workflow incorporates sample and NGS library QC using the Agilent Bioanalyzer or TapeStation systems. Library preparation can be automated using either the Agilent Magnis NGS Prep system[1] or the high-throughput Agilent Bravo NGS workstation[1] to generate sequencing-ready libraries. Compatible sequencers include the low-throughput Illumina MiSeq and MiniSeq instruments, enabling affordable tumor genomic profiling. Efficiently integrate raw read alignment, variant annotation and classification, and reporting modules using the Agilent Alissa Clinical Informatics Platform.

Table 1. SureSelect Cancer Tumor-Specific assays. The SureSelect Cancer Tumor-Specific assays include panels for different tissues. Each covers a small number of genes to enable cost-effective tumor genomic profiling.

SureSelect Cancer Tumor-Specific Assay	Size (Mb)	Number of Genes
Lung	0.42	53
Colon	0.27	35
Pancreas	0.41	58
Kidney	0.27	35
Bladder	0.25	25

[1]Altough library preparation for SureSelect Cancer Tumor-Specific assays is compatible with Magnis and Bravo automation, the assay performance specifications have not been verified.

Excellent Target Coverage and Uniform Distribution for Reliable Results

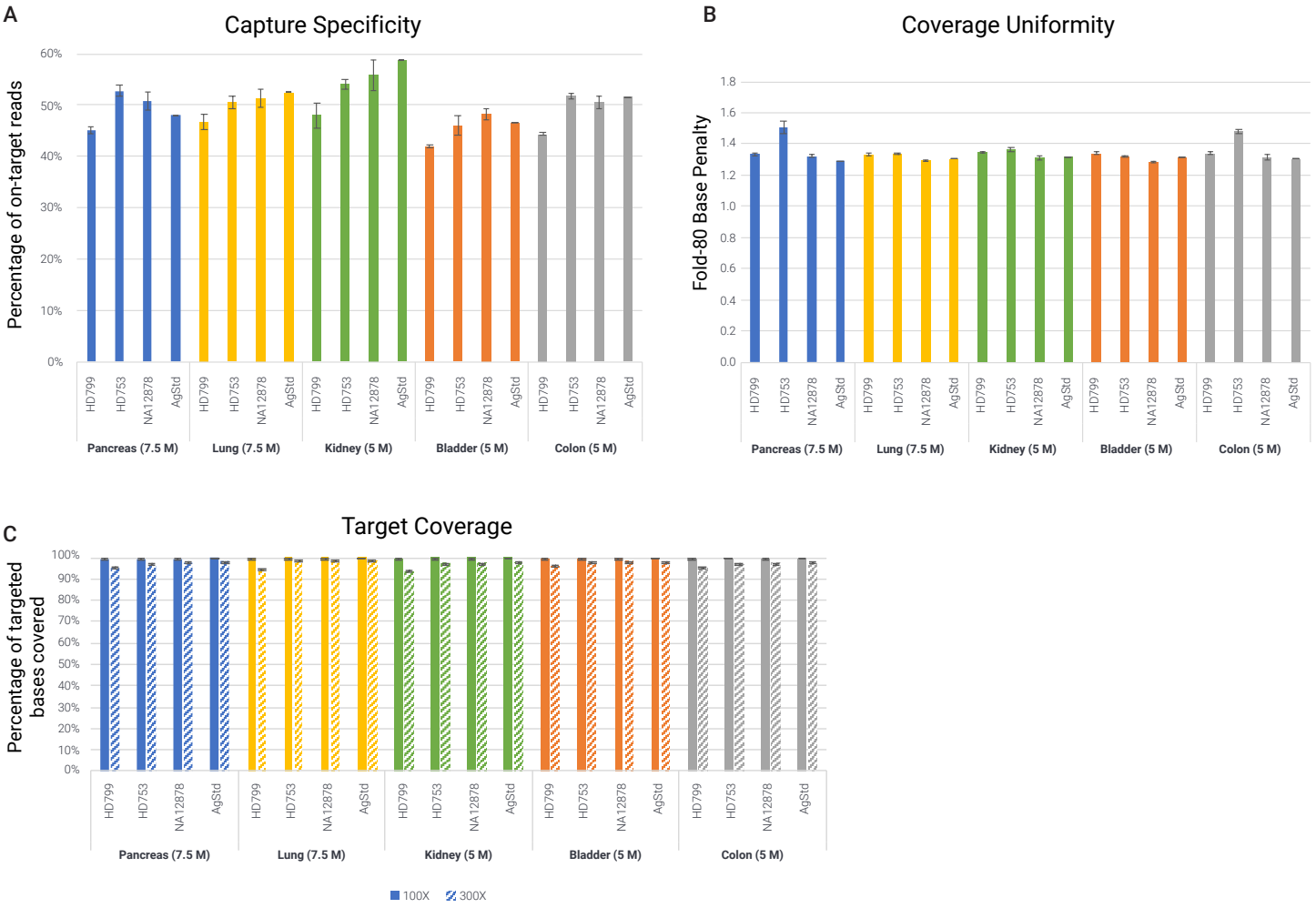

Figure 2. Superior enrichment efficiency of the Agilent SureSelect Cancer Tumor-Specific assays enable deep target coverage. **A.** The percentage of reads overlapping the target regions is shown across SureSelect Cancer Tumor-Specific assays using reference samples from Horizon and Agilent Technologies. Fifty nanograms of DNA from the indicated reference samples were processed using Agilent SureSelect XT HS2 library preparation reagents with enzymatic shearing, as recommended for the SureSelect Cancer Tumor-Specific assays and sequenced on the Illumina HiSeq4000 (2 x 150 bp reads). Data were down sampled to 7.5 M reads for the SureSelect Cancer Lung and Pancreas assays and 5 M reads for the SureSelect Cancer Colon, Kidney, and Bladder assays. Samples tested include the Horizon Discovery Quantitative Multiplex Reference Standard fcDNA (moderate, HD799); Horizon Discovery Structural Multiplex Reference Standard gDNA (HD753); Coriell Institute HapMap DNA NA12878; and Agilent OneSeq Reference DNA, Female (p/n 5190-8850, AgStd). **B.** As a measure of coverage uniformity, the fold-80 base penalty is shown for the SureSelect Cancer Tumor-Specific assays. A lower fold-80 value represents more even coverage across the targeted regions. Experimental details and samples were as described in Figure 2A. **C.** The fraction of targeted bases that have greater than or equal to 100X or 300X coverage is shown for each of the SureSelect Cancer Tumor-Specific assays. The experimental details and samples are described in Figure 2A.

Consistent Detection of Key Somatic Variant Classes

Table 2. SureSelect Cancer Tumor-Specific assays show consistent detection of single nucleotide variants (SNVs), insertions (ins.), deletions (del.), and copy number variations (CNVs) in comparison with the SureSelect Cancer CGP assay, down to 5% variant allele frequency (VAF). Fifty nanograms of the Horizon Discovery Structural Multiplex Reference Standard gDNA (HD753) was assayed and sequenced on the Illumina HiSeq4000 Sequencer (2 x 150 bp reads). The resulting sequencing reads were down sampled to 40 M reads for the SureSelect Cancer CGP assay, 7.5 M reads for the SureSelect Cancer Lung and Pancreas assays, and 5 M reads for the SureSelect Cancer Colon, Bladder, and Kidney assays. Measured allele frequency represents the average of three replicate assays. All variants were detected in all three replicates. Gray boxes represent genes that are not targeted in the respective SureSelect Cancer Tumor-Specific assay.

Gene	Variant	Variant Type	Expected Allelic Frequency	Measured Allelic Frequency					
				SureSelect Cancer CGP Assay	SureSelect Cancer Tumor-Specific Assay				
					Lung	Colon	Pancreas	Bladder	Kidney
AKT1	E17K	SNV	5.0%	5.5%	4.9%			4.8%	
BRAF	V600E	SNV	18.2%	15.8%	16.6%	16.7%	18.2%	16.3%	
BRCA2	K1691Nfs*15	1 bp del.	5.6%	4.5%			5.7%		
EGFR	G719S	SNV	5.3%	4.3%	3.8%				
EGFR	ΔE746-A750	9 bp ins.	5.3%	1.8%	2.9%				
EGFR	V769_ D770insASV	15 bp ins.	5.6%	4.0%	3.9%				
FBXW7	S668Vfs*39	1 bp del.	5.6%	4.7%		5.1%	6.1%		
GNA11	Q209L	SNV	5.6%	5.5%					
KRAS	G13D	SNV	5.6%	4.9%	5.0%	4.2%	4.7%	4.7%	
MET	L238Yfs*25	1 bp del.	2.5%	2.9%	3.1%		3.3%		3.3%
MET	Amplification	CNV	5 copies	5 copies	5 copies		5 copies		4 copies
PIK3CA	E545K	SNV	5.6%	4.6%	7.0%	5.9%	4.4%	5.6%	5.5%
PIK3CA	H1047R	SNV	16.7%	14.7%	15.1%	15.0%	16.2%	16.9%	15.3%

De Novo Gene Fusion Detection from Just One Gene Partner

Table 3. The SureSelect Cancer Lung and Cancer CGP assays provide detection of translocations from DNA inputs. The SureSelect Cancer CGP assay can detect fusions from RNA inputs as well. Four non-small cell lung cancer (NSCLC) samples with translocations involving the *ALK* gene were analyzed with either the SureSelect Cancer CGP DNA, Cancer CGP RNA, or the SureSelect Cancer Lung assay. The allele count represents the number of reads that map to the genomic position of the breakpoint when assayed with the DNA assays. The number of fusion reads is the number of reads that map to both the *ALK* and *EML4* transcripts. Detection of DNA translocation events was performed using an internally developed algorithm. Detection of the RNA fusion reads was performed using STAR-Fusion. Fifty nanograms of either DNA or RNA extracted from formalin-fixed, paraffin-embedded (FFPE) samples was used as input. Sequencing was performed on the Illumina HiSeq4000 (2 x 150 bp reads) and the resulting data were down sampled to 40 M reads for the SureSelect Cancer CGP DNA assay, 10 M reads for the SureSelect Cancer CGP RNA assay, and 7.5 M reads for the SureSelect Cancer Lung assay.

Sample ID	*ALK/EML4* Breakpoint	SureSelect Cancer CGP Assay: DNA Panel		SureSelect Cancer CGP Assay: RNA Panel		SureSelect Cancer Lung Assay: DNA Panel	
		Read Depth	Allele Count	*ALK/EML4* Fusion	Fusion Reads	Read Depth	Allele Count
NSCLC-1	chr2:29224722/42275660	256	18	Detected	8	195	14
NSCLC-2	chr2:29223615/42297197	152	9	Detected	23	126	9
NSCLC-3	chr2:29224445/42276523	154	14	Detected	15	106	8
NSCLC-4	chr2:29223466/42317566	291	61	Detected	96	273	42

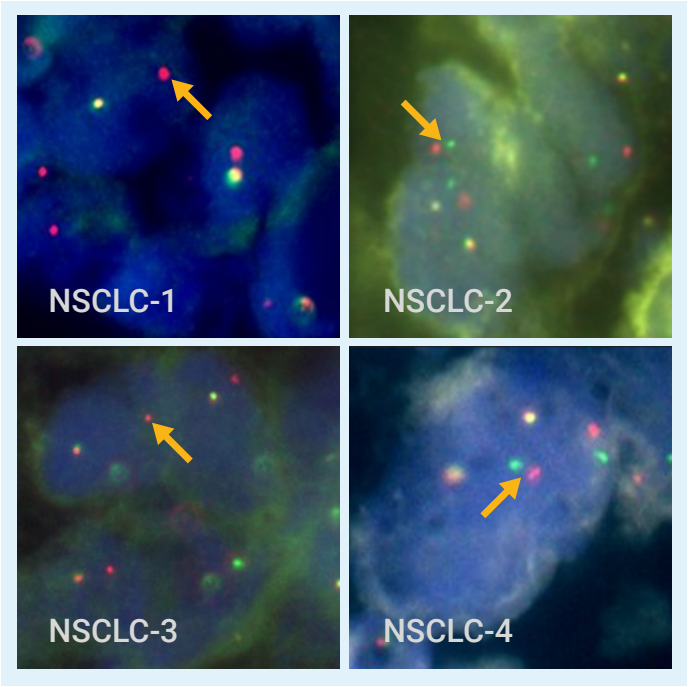

Figure 3. Representative images from fluorescent in situ hybridization (FISH) analysis of the four *ALK*-positive samples to show concordant results with NGS results in Table 3. FISH was performed using the Agilent SureFISH *ALK* Break-Apart probe. Evidence of a translocation involving *ALK* is visualized as either a lone red signal (arrow) or separation of the red and green signals (arrow).

Enabling Variant Detection from Cell-Free DNA (cfDNA) Samples

Table 4. Single nucleotide variants (SNVs) and insertions/deletions can be reliably detected in libraries made from 50 ng cfDNA reference samples using either the SureSelect Cancer CGP panel or the Cancer Tumor-Specific assays. The Horizon Discovery Multiplex I cfDNA Reference Standard Set covers multiple, engineered, SNVs with eight mutations. The table shows data for a sample with 5% variant allele frequency (VAF) (cf_HD777-5%). For the wildtype sample (cfDNA_HD776_wt), both expected and observed allelic frequencies were 0% (data not shown). This experiment used the recommended Agilent SureSelect DNA XT HS2 library construction protocol with enzymatic shearing as recommended for the SureSelect Cancer Tissue-Specific assays. Sequencing was performed on the Illumina NovaSeq6000 (2 x 150 bp reads) for the SureSelect Cancer Tumor-Specific assays, and on the Illumina HiSeq4000 for the SureSelect Cancer CGP assay. The resulting data were down sampled to 40 M reads for the SureSelect Cancer CGP DNA assay, 7.5 M reads for the SureSelect Cancer Lung and Pancreas assays, and 5 M reads for the SureSelect Cancer Kidney, Bladder and Colon assays. Measured allele frequency represents the average of duplicate samples, except for the SureSelect Cancer CGP assay which was averaged across three replicates. Gray boxes represent genes that are not targeted in the respective SureSelect Cancer Tumor-Specific assay.

Gene	Variant	Variant Type	Expected Allelic Frequency	Measured Allelic Frequency					
				SureSelect Cancer CGP Assay	SureSelect Cancer Tumor-Specific Assay				
					Lung	Colon	Pancreas	Bladder	Kidney
EGFR	L858R	SNV	5%	3%	4%				
EGFR	T790M	SNV	5%	5%	5%				
KRAS	G12D	SNV	5%	6%	5%	4%	4%	5%	
NRAS	Q61K	SNV	5%	7%	6%	6%	7%		
NRAS	A59T	SNV	5%	7%	5%	5%	8%		
PIK3CA	E545K	SNV	5%	4%	5%	6%	5%	5%	6%
EGFR	ΔE746-A750	SNV	5%	3%	3%				
EGFR	V769_D770insASV	SNV	5%	3%	3%				

Table 5. Genes targeted in each SureSelect Cancer Tumor-Specific assay.

SureSelect Cancer Lung Assay

SNVs/Indels									CNVs		Translocations
AKT1	*CCND1*	*CDK6*	*ERBB3*	*HRAS*	*KRAS*	*MSH6*	*NTRK3*	*RAF1*	*BRAF*	*EGFR*	*ALK*:[18,19]
ALK	*CCND2*	*CDKN2A*	*ERCC2*	*IDH1*	*MAP2K1*	*MTOR*	*PDGFRA*	*RET*	*CCND1*	*ERBB2*	*FGFR2*:[17]
APC	*CCNE1*	*CTNNB1*	*FGFR1*	*IDH2*	*MAP2K2*	*NF1*	*PIK3CA*	*ROS1*	*CCND2*	*FGFR1*	*FGFR3*:[17,18]
ARAF	*CD274*	*DDR2*	*FGFR2*	*KDM6A*	*MET*	*NRAS*	*PMS2*	*STK11*	*CD274*	*MET*	*NTRK1*:[8,9,10,11]
ARID1A	*CDK12*	*EGFR*	*FGFR3*	*KEAP1*	*MLH1*	*NTRK1*	*PTCH1*	*TP53*	*CDK6*	*PIK3CA*	*RET*:[7,10,11]
BRAF	*CDK4*	*ERBB2*	*FGFR4*	*KIT*	*MSH2*	*NTRK2*	*PTEN*		*CDKN2A*	*PTEN*	*ROS1*:[31,33,34,35]

SureSelect Cancer Colon Assay

SNVs/Indels									CNVs		Translocations
APC	*BRAF*	*CTNNB1*	*FBXW7*	*MLH1*	*MUTYH*	*PIK3CA*	*PTEN*	*STK11*	*CDKN2A*	*PIK3CA*	*BRAF*:[8,9,10]
ARID1A	*CDH1*	*EPCAM*	*GNAS*	*MSH2*	*MYC*	*PMS2*	*RNF43*	*TCF7L2*	*ERBB2*	*PTEN*	
AXIN2	*CDKN2A*	*ERBB2*	*GREM1*	*MSH3*	*NRAS*	*POLD1*	*SMAD4*	*TP53*	*KRAS*		
BMPR1A	*CHEK2*	*ERBB3*	*KRAS*	*MSH6*	*NTHL1*	*POLE*	*SOX9*		*MYC*		

SureSelect Cancer Pancreas Assay

SNVs/Indels									CNVs	Translocations
ALK	*BRCA1*	*CPA1*	*FBXW7*	*MDM2*	*NF1*	*POLD1*	*SMAD4*	*TSC2*	*BRCA1*	*ALK*:[18,19]
APC	*BRCA2*	*CTNNB1*	*FGFR2*	*MEN1*	*NRAS*	*POLE*	*SOX9*	*VHL*	*BRCA2*	*FGFR2*:[17]
ARID1A	*CASR*	*CTRC*	*GNAS*	*MET*	*NRG1*	*PRSS1*	*SPINK1*		*ERBB2*	*NTRK1*:[8,9,10,11]
ATM	*CDH1*	*EPCAM*	*IDH1*	*MLH1*	*NTRK1*	*PTEN*	*STK11*		*KRAS*	*RET*:[7,10,11]
BAP1	*CDKN2A*	*ERBB2*	*IDH2*	*MSH2*	*PALB2*	*RET*	*TCF7L2*		*MDM2*	*ROS1*:[31,33,34,35]
BMPR1A	*CFTR*	*ERBB3*	*KRAS*	*MSH6*	*PIK3CA*	*RNF43*	*TP53*		*MET*	*ROS1*:[31,33,34,35]
BRAF	*CHEK2*	*ESR1*	*MAP2K1*	*MYC*	*PMS2*	*ROS1*	*TSC1*		*MYC*	

SureSelect Cancer Kidney Assay

SNVs/Indels									CNVs
ATM	*DICER1*	*FLCN*	*MLH1*	*NF2*	*PTEN*	*SDHC*	*SMARCB1*	*TSC2*	*MET*
BAP1	*DIS3L2*	*GPC3*	*MSH2*	*PBRM1*	*REST*	*SDHD*	*TFEB*	*VHL*	*PIK3CA*
CDC73	*EPCAM*	*KDM5C*	*MSH6*	*PIK3CA*	*SDHA*	*SETD2*	*TP53*	*WT1*	*PTEN*
CDKN1C	*FH*	*MET*	*MTOR*	*PMS2*	*SDHB*	*SMARCA4*	*TSC1*		

SureSelect Cancer Bladder Assay

SNVs/Indels							CNVs		Translocations
AKT1	*CCND1*	*CTNNB1*	*ERCC2*	*KDM6A*	*PPARG*	*TSC1*	*CCND1*	*KRAS*	*BRAF*:[8,9,10]
ARID1A	*CCNE1*	*E2F3*	*FGFR2*	*KRAS*	*PTEN*		*CCNE1*	*MDM2*	*FGFR2*:[17]
ATM	*CDKN1A*	*ERBB2*	*FGFR3*	*MDM2*	*RB1*		*CDKN2A*	*PIK3CA*	*FGFR3*:[17,18]
BRAF	*CDKN2A*	*ERBB3*	*HRAS*	*PIK3CA*	*TP53*		*ERBB2*	*PTEN*	

Ordering Information

SureSelect Cancer Tumor-Specific Assays–Probes Only		
Product Description	**16 Rxns**	**96 Rxns Auto***
SureSelect Cancer Lung Assay Probe, DNA	5282-0060	5282-0061
SureSelect Cancer Colon Assay Probe, DNA	5282-0062	5282-0063
SureSelect Cancer Pancreas Assay Probe, DNA	5282-0064	5282-0065
SureSelect Cancer Kidney Assay Probe, DNA	5282-0066	5282-0067
SureSelect Cancer Bladder Assay Probe, DNA	5282-0068	5282-0069
SureSelect Cancer Tumor-Specific Assays–Manual Library Preparation and Target Enrichment Kits		
Product Description	**16 Rxns**	**96 Rxns**
SureSelect XT HS2 DNA Starter kit with index primer pairs 1 to 16**	G9982A	
SureSelect XT HS2 DNA Reagent kit with AMPure XP/Streptavidin Beads and index primer pairs 1 to 96		G9984A
SureSelect Enzymatic Fragmentation kit		5191-4080
SureSelect Cancer Tumor-Specific–Automated Library Preparation and Target Enrichment Kits		
Product Description		**96 Rxns Auto**
Magnis SureSelect XT HS2 DNA, No Probe, ILMN**		G9750B
SureSelect XT HS2 DNA Library Preparation kit with index primer pairs 1 to 96		G9985A
SureSelect Enzymatic Fragmentation kit, auto		5191-6764
SureSelect Cancer CGP RNA Assay–Manual Library Preparation and Target Enrichment Kits		
Product Description	**16 Rxns**	**96 Rxns**
SureSelect Cancer CGP Assay RNA kit	G9968A	G9968B
SureSelect Enzymatic Fragmentation kit	5191-4079	5191-4080
SureSelect Cancer CGP RNA Assay–Automation Library Preparation and Target Enrichment Kits		
Product Description	**32 Rxns Auto**	**96 Rxns Auto**
Magnis SureSelect Cancer CGP XT HS2 RNA kit	G9777C	G9777D
SureSelect Cancer CGP Assay RNA kit, auto		G9968C
SureSelect Enzymatic Fragmentation kit, auto		5191-6764

**96 reactions kits are compatible with both manual and automation workflows.*
***Enzymatic fragmentation reagents included.*

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