

## VARIANT*Plex*-HT Complete Solid Tumor

### Description

The VARIANT*Plex*-HT Complete Solid Tumor panel is a balanced pool of gene-specific primer (GSP) oligonucleotides that is optimized for use with VARIANT*Plex*-HT reagents and molecular barcode (MBC) adapters to produce targeted NGS libraries. This product insert should be used in conjunction with VARIANT*Plex*-HT protocol for Illumina® (RA-DOC-058).

VARIANT*Plex*-HT Complete Solid Tumor contains **19,943** GSPs targeting **430** genes commonly mutated in solid tumor cancer types as well as microsatellite instability (**MSI**) and tumor mutational burden (**TMB**) status.

Description	Part number	Storage
VARIANT <i>Plex</i> -HT Complete Solid Tumor GSP1, 24 reactions or VARIANT <i>Plex</i> -HT Complete Solid Tumor GSP1, 96 reactions	SA22534241 or SA22534961	-20°C ± 10°C
VARIANT <i>Plex</i> -HT Complete Solid Tumor GSP2, 24 reactions or VARIANT <i>Plex</i> -HT Complete Solid Tumor GSP2, 96 reactions	SA22534242 or SA22534962	

### Required reagent volumes

Protocol reference	Protocol step	Reagent	Volume per reaction (µL)
A	Ligation Step 2 Elution	5mM NaOH	20
B	First PCR	VARIANT <i>Plex</i> -HT Complete Solid Tumor GSP1	8
C	First PCR	10mM Tris-HCl pH 8.0	18
D	First PCR	Purified PCR1 eluate	16
E	Second PCR	VARIANT <i>Plex</i> -HT Complete Solid Tumor GSP2	8

## Recommended PCR cycling

	Step	Temperature (°C)	Time	Cycles
First PCR reaction	1	95	3 min	1
	2	95	30 sec	
	3	58	10 sec	15
	4	60	10 min (100% ramp rate)	
	5	72	3 min	1
	6	4	Hold	1
Second PCR reaction	1	95	3 min	1
	2	95	30 sec	
	3	58	10 sec	20 <sup>†</sup>
	4	65	10 min (100% ramp rate)	
	5	72	3 min	1
	6	4	Hold	1

†The number of PCR2 cycles may be decreased if you regularly experience library yields greater than 200 nM.

## Recommended reads and multiplexing

VARIANT*Plex*-HT Complete Solid Tumor libraries should be sequenced to a minimum of **45M** reads. Starting read depth recommendations for standard profiling may be adjusted based on user needs.

## Archer™ Analysis settings

Sequencing data should be processed using Archer Analysis (v7.2, or greater). The VARIANT*Plex*-HT Complete Solid Tumor panel is compatible with the **SNV/Indel, Copy Number Variation, Structural Variation, MSI, and TMB** pipelines, found under the **DNA** Input Type. The recommended TMB settings in Archer Analysis are *High Threshold for TMB* = 17.5 and *Low Threshold for TMB* = 6 for VARIANT*Plex* Complete Solid Tumor. As with most Analysis settings, these may be adjusted based on user needs. Selection of the DNA

Target Coverage pipeline is optional (see the Archer Analysis User Guide for more details on setting up your analysis).

Archer Analysis processing of VARIANT $Pl$ ex-HT Complete Solid Tumor libraries requires a one-time upload of the Panel GTF. When performing DNA Target Coverage analysis, users must also select a Region of Interest BED file. Users may optionally add a Targeted Mutations VCF file for targeted SNV/Indel detection. Files can be obtained by contacting [archer-tech@idtdna.com](mailto:archer-tech@idtdna.com)

## Assay targets

### Integrative genomic biomarkers

MSI, TMB

### Genes

The following genes in the panel have full coding sequence coverage for SNV/Indel analysis. Coding sequence was determined using NCBI RefSeq transcripts<sup>†</sup>. These genes are also enabled for Copy Number Variation (CNV) analysis.

<i>ABL1</i>	<i>CCND1</i>	<i>EIF1AX</i>	<i>FLT4</i>	<i>KANSL1</i>	<i>MST1R</i>	<i>PLCB4</i>	<i>RPL5</i>	<i>TFRC</i>
<i>ABL2</i>	<i>CCND2</i>	<i>ELF3</i>	<i>FOXA1</i>	<i>KDM5A</i>	<i>MTOR</i>	<i>PLCG1</i>	<i>RPS6KB1</i>	<i>TGFBR1</i>
<i>ACVR1</i>	<i>CCND3</i>	<i>EP300</i>	<i>FOXA2</i>	<i>KDM5C</i>	<i>MUC16</i>	<i>PLXNB2</i>	<i>RPTOR</i>	<i>TGFBR2</i>
<i>ACVR2A</i>	<i>CCNE1</i>	<i>EPAS1</i>	<i>FOXL2</i>	<i>KDM6A</i>	<i>MUC6</i>	<i>PMS1</i>	<i>RRAS2</i>	<i>TGIF1</i>
<i>AKT1</i>	<i>CD274 (PD-L1)</i>	<i>EPCAM</i>	<i>FOXO1</i>	<i>KDR</i>	<i>MUTYH</i>	<i>PMS2</i>	<i>RUNX1</i>	<i>THRAP3</i>
<i>AKT2</i>	<i>CD70</i>	<i>EPHA2</i>	<i>FOXP1</i>	<i>KEAP1</i>	<i>MYC</i>	<i>POLD1</i>	<i>RUNX1T1</i>	<i>TLR4</i>
<i>AKT3</i>	<i>CD79A</i>	<i>EPHA3</i>	<i>FOXQ1</i>	<i>KEL</i>	<i>MYCL (MYCL1)</i>	<i>POLE</i>	<i>RXRA</i>	<i>TMSB4X</i>
<i>ALK</i>	<i>CD79B</i>	<i>EPHA7</i>	<i>FUBP1</i>	<i>KIF1A</i>	<i>MYCN</i>	<i>POLQ</i>	<i>SCAF4</i>	<i>TNFAIP3</i>
<i>APC</i>	<i>CDC73</i>	<i>EPHB1</i>	<i>GATA1</i>	<i>KIT*</i>	<i>MYD88</i>	<i>POLRMT</i>	<i>SDHA</i>	<i>TNFRSF14</i>
<i>AR</i>	<i>CDH1</i>	<i>ERBB2</i>	<i>GATA2</i>	<i>KLF4</i>	<i>MYH9</i>	<i>PPARG</i>	<i>SDHB</i>	<i>TP53</i>
<i>ARAF</i>	<i>CDK12</i>	<i>ERBB3</i>	<i>GATA3</i>	<i>KLF5</i>	<i>NBN</i>	<i>PPM1D</i>	<i>SDHC</i>	<i>TP63</i>
<i>ARHGAP35</i>	<i>CDK4</i>	<i>ERBB4</i>	<i>GEN1</i>	<i>KMT2A (MLL)</i>	<i>NCOR1</i>	<i>PPP2R1A</i>	<i>SDHD</i>	<i>TRAF3</i>
<i>ARID1A</i>	<i>CDK6</i>	<i>ERCC1</i>	<i>GLI1</i>	<i>KMT2B</i>	<i>NF1</i>	<i>PPP2R2A</i>	<i>SETBP1</i>	<i>TRAF7</i>
<i>ARID1B</i>	<i>CDK8</i>	<i>ERCC2</i>	<i>GNA11</i>	<i>KMT2C</i>	<i>NF2</i>	<i>PPP6C</i>	<i>SETD2</i>	<i>TSC1</i>
<i>ARID2</i>	<i>CDKN1A</i>	<i>ERCC4</i>	<i>GNA13</i>	<i>KMT2D (MLL2)</i>	<i>NFE2L2</i>	<i>PRDM1</i>	<i>SF3B1</i>	<i>TSC2</i>

ARID5B	CDKN1B	ERG	GNAQ	KRAS	NFKBIA	PRKAR1A	SH2D1A	TSHR
ASXL1	CDKN2A	ERRFI1	GNAS	KRT222	NIPBL	PRKD1	SLX4	TXNIP
ASXL2	CDKN2B	ESR1	GPS2	LAMP1	NKX2-1	PRKDC	SMAD2	U2AF1
ATM*	CDKN2C	EWSR1	GRIN2D	LATS1	NOTCH1*	PSIP1	SMAD3	UNCX
ATR	CEBPA	EZH2	GRM3	LATS2	NOTCH2	PTCH1	SMAD4	USP9X
ATRX	CHD1	FAM175A	H3F3A	LEMD2	NOTCH3	PTEN	SMARCA4	VHL
AURKA	CHD3	FAM46C	H3F3B	LRP1B	NOTCH4	PTMA	SMARCB1	WT1
AURKB	CHD4	FAM46D	H3F3C	LZTR1	NPM1	PTPDC1	SMC1A	XPO1
AXIN1	CHD8	FANCA	HGF	MACF1	NRAS	PTPN11	SMC3	XRCC2
AXIN2	CHEK1	FANCC	HIST1H1C	MAP2K1 (MEK1)	NSD1	PTPRC	SMO	XRCC3
AXL	CHEK2*	FANCD2	HIST1H1E	MAP2K2 (MEK2)	NTRK1	PTPRD	SOCS1	ZBTB20
B2M	CIC	FANCE	HIST1H2BD	MAP2K4	NTRK2	PTPRT	SOX17	ZFHX3
BAP1	COL5A1	FANCF	HIST1H3B	MAP3K1	NTRK3	RAC1	SOX2	ZMYM2
BARD1	CREBBP	FANCG	HIST1H3C	MAP3K13	NUP93	RAD21	SOX9	ZMYM3
BCL2	CRKL	FANCI	HNF1A	MAP3K4	PALB2	RAD50	SPEN	ZNF750
BCL2L1	CSDE1	FANCL	HOXB13	MAPK1	PARP1	RAD51	SPOP	
BCL2L11	CSF1R	FAT1	HRAS	MAX	PAX5	RAD51B	SPTA1	
BCL6	CSF3R	FBXW7	HUWE1	MCL1	PAX8	RAD51C	SPTAN1	
BCOR	CTCF	FGF1	IDH1	MDM2	PBRM1	RAD51D	SRC	
BCORL1	CTLA4	FGF19	IDH2	MDM4	PDCD1 (PD-1)	RAD52	SRSF2	
BIRC3	CTNNA1	FGF2	IGF1R	MECOM	PDCD1LG2 (PD-L2)	RAD54L	STAG1	
BLM	CTNNB1*	FGF3	IKBKE	MED12	PDGFRA*	RAF1	STAG2	
BMPR1A	CUL3	FGF4	IKZF1	MEF2B	PDGFRB	RARA	STAT3	
BRAF*	CYSLTR2	FGF7	IL6ST	MEN1	PGR	RASA1	STK11	
BRCA1*	DAXX	FGF8	IL7R	MET*	PHF6	RB1	SUFU	
BRCA2*	DDR2	FGF9	INPP4B	MGA	PIK3C2B	RBM10	SYK	
BRIP1	DDX3X	FGFR1	INPPL1	MGMT	PIK3C2G	RECQL4	TAF1	
BTG1	DICER1	FGFR2	IRF2	MITF	PIK3CA	REL	TBL1XR1	
BTG2	DMD	FGFR3	IRF4	MLH1	PIK3CB	RET*	TBX3	
BTK	DNMT3A	FGFR4	IRF6	MLLT3	PIK3CD	RHEB	TCEB1	
CACNA1A	DOT1L	FH	IRS2	MPL	PIK3CG	RHOA	TCF3	

<i>CARD11</i>	<i>EEF1A1</i>	<i>FLCN</i>	<i>JAK1</i>	<i>MRE11A</i>	<i>PIK3R1*</i>	<i>RICTOR</i>	<i>TCF7L2</i>
<i>CASP8</i>	<i>EEF2</i>	<i>FLNA</i>	<i>JAK2</i>	<i>MSH2</i>	<i>PIK3R2</i>	<i>RIT1</i>	<i>TERT</i>
<i>CBFB</i>	<i>EGFR</i>	<i>FLT1</i>	<i>JAK3</i>	<i>MSH3</i>	<i>PIK3R3</i>	<i>RNF43</i>	<i>TET1</i>
<i>CBL</i>	<i>EGR3</i>	<i>FLT3</i>	<i>JUN</i>	<i>MSH6</i>	<i>PIM1</i>	<i>ROS1</i>	<i>TET2</i>

†Contact [adx-tech@idtdna.com](mailto:adx-tech@idtdna.com) for the panel target file to view complete list of targeted regions.

\*Indicates that select regions of this gene are enabled for Structural Variant analysis in the GTF.

## SNPs and sites targeted for sample tracking

rs560681	rs430046	rs987640	rs10776839	rs12393891
rs740598	rs8078417	rs6444724	rs6530357	chrX:4429309
rs1498553	rs9951171	rs6811238	rs5971553	chrX:11314433
rs10773760	rs576261	rs13182883	rs5953060	chrY:6738552
rs1058083	rs1109037	rs214955	rs6524626	chrY:19490214
rs4530059	rs1523537	rs321198	rs5940270	
rs1821380	rs221956	rs4606077	rs722847	

SNPs may be used in combination to uniquely tag and track samples over time. Contact [archer-tech@idtdna.com](mailto:archer-tech@idtdna.com) for further details.

## Limitations of use

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## Revision History

Document Number	Date	Description of change
<i>RA-DOC-062/REV01</i>	October 2023	Initial release.
<i>RA-DOC-062/REV02</i>	November 2023	Updated First and Second PCR cycling conditions to include separate anneal and extended steps.  Updated branding.