# VARIANT*Plex*-HT Pan Solid Tumor

## Description

The VARIANT*Plex*-HT Pan 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 Pan Solid Tumor contains **9878** GSPs targeting **185** genes commonly mutated in solid tumors as well as microsatellite instability (**MSI**) and tumor mutational burden (**TMB**) status.

| Description | Part number | Storage |
|---|---|---|
| VARIANT*Plex*-HT Pan Solid Tumor GSP1, 24 reactions<br>or<br>VARIANT*Plex*-HT Pan Solid Tumor GSP1, 96 reactions | SA20196241<br>or<br>SA20196961 | –20°C ± 10°C |
| VARIANT*Plex*-HT Pan Solid Tumor GSP2, 24 reactions<br>or<br>VARIANT*Plex*-HT Pan Solid Tumor GSP2, 96 reactions | SA20196242<br>or<br>SA20196962 | |

## Required reagent volumes

| Protocol reference | Protocol step | Reagent | Volume per reaction (µL) |
|---|---|---|---|
| A | Ligation Step 2 Elution | 5mM NaOH | 20 |
| B | First PCR | VARIANT*Plex*-HT Pan 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*Plex*-HT Pan Solid Tumor GSP2 | 8 |

## Recommended PCR cycling

| | Step | Temperature (°C) | Time | Cycles |
|---|---|---|---|---|
| **First PCR reaction** | **1** | 95 | 3 min | 1 |
| | **2** | 95 | 30 sec | 15 |
| | **3** | 58 | 10 sec | |
| | **4** | 60 | 10 min<br>(100% ramp rate) | |
| | **5** | 72 | 3 min | 1 |
| | **6** | 4 | Hold | 1 |

| | Step | Temperature (°C) | Time | Cycles |
|---|---|---|---|---|
| **Second PCR reaction** | **1** | 95 | 3 min | 1 |
| | **2** | 95 | 30 sec | 20[†] |
| | **3** | 58 | 10 sec | |
| | **4** | 65 | 10 min<br>(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 Pan Solid Tumor libraries should be sequenced to a minimum of **25M** 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 Pan Solid Tumor panel works with selection of 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* = 20 and *Low Threshold* for TMB = 5 for VARIANT*Plex* Pan 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*Plex*-HT Pan 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

# 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†.

| | | | | | | |
|---|---|---|---|---|---|---|
| *ABL1* | *BMPR1A* | *CTNNB1** | *FGF19* | *HNF1A* | *MDM4* | *NOTCH2* |
| *ACVR1* | *BRAF** | *DAXX* | *FGFR1* | *HRAS* | *MED12* | *NOTCH3* |
| *AKT1* | *BRCA1** | *DDR2* | *FGFR2* | *IDH1* | *MEN1* | *NOTCH4* |
| *AKT2* | *BRCA2** | *DDX3X* | *FGFR3* | *IDH2* | *MET** | *NPM1* |
| *AKT3* | *BRIP1* | *DICER1* | *FGFR4* | *JAK1* | *MLH1* | *NRAS* |
| *ALK* | *CCND1* | *EGFR* | *FH* | *JAK2* | *MPL* | *NTRK1* |
| *APC* | *CCND2* | *EIF1AX* | *FLCN* | *JAK3* | *MRE11A* | *NTRK2* |
| *AR* | *CCND3* | *EP300* | *FLT1* | *KDM6A* | *MSH2* | *NTRK3* |
| *ARID1A* | *CCNE1* | *EPCAM* | *FLT3* | *KDR* | *MSH3* | *PALB2* |
| *ARID1B* | *CDH1* | *ERBB2* | *FLT4* | *KEAP1* | *MSH6* | *PBRM1* |
| *ARID2* | *CDK12* | *ERBB3* | *FOXA1* | *KIT** | *MTOR* | *PDGFRA** |
| *ATM** | *CDK4* | *ERBB4* | *FOXL2* | *KLF4* | *MUC16* | *PIK3CA* |
| *ATR* | *CDK6* | *ERCC1* | *FUBP1* | *KMT2C* | *MUTYH* | *PIK3CB* |
| *ATRX* | *CDKN2A* | *ERCC2* | *GNA11* | *KMT2D (MLL2)* | *MYC* | *PIK3R1** |
| *AURKA* | *CDKN2B* | *ESR1* | *GNAQ* | *KRAS* | *MYCN* | *PLCB4* |
| *B2M* | *CHD1* | *EZH2* | *GNAS* | *LZTR1* | *NBN* | *PMS2* |
| *BAP1* | *CHEK1* | *FANCA* | *H3F3A* | *MAP2K1 (MEK1)* | *NF1* | *POLD1* |
| *BARD1* | *CHEK2** | *FANCI* | *H3F3B* | *MAP2K2 (MEK2)* | *NF2* | *POLE* |
| *BCOR* | *CIC* | *FANCL* | *HIST1H3B* | *MAP3K1* | *NKX2-1* | *PPP2R1A* |

| | | | | | | |
|---|---|---|---|---|---|---|
| BLM | CSF1R | FBXW7 | HIST1H3C | MDM2 | NOTCH1* | PPP2R2A |
| PRKD1 | RAD51C | RICTOR | SETD2 | SRC | TP53 | VHL |
| PTCH1 | RAD51D | RNF43 | SF3B1 | SRSF2 | TP63 | XRCC2 |
| PTEN | RAD54L | ROS1 | SMAD2 | STAG2 | TRAF7 | XRCC3 |
| PTPN11 | RAF1 | SDHA | SMAD4 | STK11 | TSC1 | |
| RAD50 | RB1 | SDHB | SMARCA4 | SUFU | TSC2 | |
| RAD51 | RET* | SDHC | SMARCB1 | TERT | TSHR | |
| RAD51B | RHOA | SDHD | SMO | TGFBR2 | U2AF1 | |

†Contact 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.

## Genes targeted for CNV

| | | | | |
|---|---|---|---|---|
| AKT1 | BRIP1 | ERBB2 | JAK2 | PDGFRA |
| AKT2 | CCND1 | ERBB3 | KDR | PIK3CA |
| AKT3 | CCND2 | ERCC1 | KEAP1 | PIK3CB |
| ALK | CCND3 | ERCC2 | KIT | PTEN |
| AR | CCNE1 | ESR1 | KRAS | RAD51B |
| ATM | CDK12 | FGF19 | MDM2 | RAF1 |
| AURKA | CDK4 | FGFR1 | MDM4 | RET |
| BARD1 | CDK6 | FGFR2 | MET | RICTOR |
| BRAF | CHEK1 | FGFR3 | MYC | STK11 |
| BRCA1 | CHEK2 | FGFR4 | MYCN | TERT |
| BRCA2 | EGFR | FLT3 | NRAS | |

Please contact archer-tech@idtdna.com to inquire about enabling additional genes for CNV detection.

## 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 for further details.

# Limitations of use

**For research use only. Not for use in diagnostic procedures**. Unless otherwise agreed to in writing, IDT does not intend these products to be used in clinical applications and does not warrant their fitness or suitability for any clinical diagnostic use. Purchaser is solely responsible for all decisions regarding the use of these products and any associated regulatory or legal obligations.

Safety data sheets pertaining to this product are available upon request.

# Revision History

| Document Number | Date | Description of change |
|---|---|---|
| *RA-DOC-065/REV01* | October 2023 | Initial release. |
| *RA-DOC-065/REV02* | November 2023 | Updated First and Second PCR cycling conditions to include separate anneal and extended steps.<br>Updated branding. |