VARIANT*Plex* Solid Tumor Focus v2

Description

The VARIANT*Plex* Solid Tumor Focus v2 panel is a balanced pool of gene-specific primer (GSP) oligonucleotides that is optimized for use with VARIANT*Plex* reagents and molecular barcode (MBC) adapters to produce targeted NGS libraries. This product insert should be used in conjunction with VARIANT*Plex* HS/HGC protocol for Illumina® (RA-DOC-056).

VARIANT*Plex* Solid Tumor Focus v2 contains **575** GSPs targeting **20** genes commonly mutated in solid tumors as well as microsatellite instability (**MSI**).

Description	Part number	Storage
VARIANT*Plex* Solid Tumor Focus v2 GSP1, 8 reactions	SA20121081	–20°C ± 10°C
VARIANT*Plex* Solid Tumor Focus v2 GSP2, 8 reactions	SA20121082	
PreSeq™ DNA QC Assay Standard, 32 µL	SA0597	
PreSeq™ DNA QC Assay 10X Primer Mix, 120 µL	SA0598	

Required reagent volumes

Protocol reference	Protocol step	Reagent	Volume per reaction (µL)
A	Ligation Step 2 Elution	5mM NaOH	36
B	First PCR	VARIANT*Plex* Solid Tumor Focus v2 GSP1	4
C	First PCR	10mM Tris-HCl pH 8.0	38
D	First PCR	Purified PCR1 eluate	36
E	Second PCR	VARIANT*Plex* Solid Tumor Focus v2 GSP2	4

Recommended PCR cycling

	Step	Temperature (°C)	Time	Cycles
First PCR reaction	**1**	95	3 min	1
	2	95	30 sec	15
	3	60	10 sec	
	4	65	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	20†
	3	60	10 sec	
	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* Solid Tumor Focus v2 libraries should be sequenced to a minimum of **1.5M 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 (v7.0, or greater). The VARIANT*Plex* Solid Tumor Focus v2 panel requires selection of the ***SNV/Indel, Structural Variation, Copy Number Variation, and MSI*** pipelines, found under the ***DNA*** Input Type (see the Archer Analysis User Guide for more details on setting up your analysis). Selection of the DNA Target Coverage pipeline is optional.

Processing of VARIANT*Plex* Solid Tumor Focus v2 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

Gene	Accession	Exon
AKT1	NM_005163	2,3,6,11
BRAF	NM_004333	11,15
EGFR	NM_005228	3,7,12,15,18,19,20,21,22
EGFR	NM_201282	16
EGFR	NM_201283	10
ERBB2	NM_004448	8,10,17,19,20,21,22,24
FOXL2	NM_023067	1(p.C134)
GNA11	NM_002067	5
GNAQ	NM_002072	4,5
GNAS	NM_000516	6,7,8,9
HRAS	NM_005343	2,3
IDH1	NM_005896	3,4
IDH2	NM_002168	4
KIT	NM_000222	2,8,9,10,11,12,13,14,15,17,18
KRAS	NM_004985	2,3,4,5
MET	NM_000245	2,11,14,15,16,19,20,21
NRAS	NM_002524	2,3,4,5
PDGFRA	NM_006206	7,10,11,12,14,15,16,18,23
PIK3CA	NM_006218	2,3,5,7,8,9,10,14,19,21

Gene	Accession	Exon
RET	NM_020630	10,11,13,14,15,16
TERT	NM_198253	3,6,10
TERT	NM_198253	Promoter (chr5:1295148-1295374)
TP53	NM_000546	1,2,3,4,5,6,7,8,9,10,11
TP53	NM_001276696	10

Genes targeted for CNV

AKT1	*EGFR*	*KIT*	*MET*	*PDGFRA*	*RET*
BRAF	*ERBB2*	*KRAS*	*NRAS*	*PIK3CA*	*TERT*

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-032/REV01	June 2023	Initial release.
RA-DOC-032/REV02	November 2023	Updated First and Second PCR cycling conditions to include separate anneal and extended steps. Added MSI pipeline information to the “Archer Analysis settings” section. Updated branding.