

VARIANT*Plex*-HT AML Focus

Description

The VARIANT*Plex*-HT AML Focus 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 AML Focus contains **226** GSPs targeting **11** genes commonly mutated in Acute Myeloid Leukemia.

Description	Part number	Storage
VARIANT <i>Plex</i> -HT AML Focus GSP1, 24 reactions or VARIANT <i>Plex</i> -HT AML Focus GSP1, 96 reactions	dSA09192241 or dSA09192961	-20°C ± 10°C
VARIANT <i>Plex</i> -HT AML Focus GSP2, 24 reactions or VARIANT <i>Plex</i> -HT AML Focus GSP2, 96 reactions	dSA09192242 or dSA09192962	

Required reagent volumes

Protocol reference	Protocol step	Reagent	Volume per reaction (µL)
A	Ligation Step 2 Elution	5mM NaOH	24
B	First PCR	VARIANT <i>Plex</i> -HT AML Focus GSP1	4
C	First PCR	10mM Tris-HCl pH 8.0	22
D	First PCR	Purified PCR1 eluate	20
E	Second PCR	VARIANT <i>Plex</i> -HT AML Focus GSP2	4

Recommended PCR cycling

	Step	Temperature (°C)	Time	Cycles
First PCR reaction	1	95	3 min	1
	2	95	30 sec	
	3	60	10 sec	15
	4	65	5 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	60	10 sec	20†
	4	65	5 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 AML Focus libraries should be sequenced to a minimum of **800,000** 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*-HT AML Focus panel requires selection of the **SNV/Indel and Structural variation** 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*-HT AML Focus 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
<i>ASXL1</i>	NM_015338	11,12,13
<i>CEBPA</i>	NM_004364	1
<i>DNMT3A</i>	NM_022552	2,3,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23
<i>DNMT3A</i>	NM_153759	1,2
<i>DNMT3A</i>	NM_175630	4
<i>FLT3</i>	NM_004119	8,9,10,11,12,13,14,15,16,17,19,20,21
<i>IDH1</i>	NM_005896	3,4
<i>IDH2</i>	NM_002168	4,6
<i>JAK2</i>	NM_004972	12,13,14,15,16
<i>KIT</i>	NM_000222	2,8,9,10,11,12,13,14,15,17,18
<i>NPM1</i>	NM_002520	11
<i>RUNX1</i>	NM_001754	2,3,5,6,7,8,9
<i>RUNX1</i>	NM_001122607	1,5
<i>TP53</i>	NM_000546	1,2,3,4,5,6,7,8,9,10,11
<i>TP53</i>	NM_001276696	10
<i>TP53</i>	NM_001276695	10

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

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

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Revision History

Document Number	Date	Description of change
<i>RA-DOC-457/REV01</i>	October 2023	Initial release
<i>RA-DOC-457/REV02</i>	November 2023	Updated First and Second PCR cycling conditions to include separate anneal and extended steps. Updated branding.