INTEGRATED DNA TECHNOLOGIES
ARCHER™

# Product Insert

VARIANT*Plex*™-HT AML Focus panel

# 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*Plex*-HT AML Focus GSP1, 24 reactions<br>or<br>VARIANT*Plex*-HT AML Focus GSP1, 96 reactions | dSA09192241<br>or<br>dSA09192961 | –20°C ± 10°C |
| VARIANT*Plex*-HT AML Focus GSP2, 24 reactions<br>or<br>VARIANT*Plex*-HT AML Focus GSP2, 96 reactions | dSA09192242<br>or<br>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*Plex*-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*Plex*-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 | 15 |
| | **3** | 60 | 10 sec | |
| | **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 | 20† |
| | **3** | 60 | 10 sec | |
| | **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 |
|---|---|---|
| *ASXL1* | NM_015338 | 11,12,13 |
| *CEBPA* | NM_004364 | 1 |
| *DNMT3A* | NM_022552 | 23 |
| *FLT3* | NM_004119 | 8,9,10,11,12,13,14,15,16,17,19,20,21 |
| *IDH1* | NM_005896 | 3,4 |
| *IDH2* | NM_002168 | 4,6 |
| *JAK2* | NM_004972 | 12,13,14,15,16 |
| *KIT* | NM_000222 | 2,8,9,10,11,12,13,14,15,17,18 |
| *NPM1* | NM_002520 | 11 |
| *RUNX1* | NM_001754 | 2,3,5,6,7,8,9 |
| *RUNX1* | NM_001122607 | 1,5 |
| *TP53* | NM_000546 | 1,2,3,4,5,6,7,8,9,10,11 |
| *TP53* | NM_001276696 | 10 |
| *TP53* | 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

**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-457/REV01* | October 2023 | Initial release |
| *RA-DOC-457/REV02* | November 2023 | Updated First and Second PCR cycling conditions to include separate anneal and extended steps.<br><br>Updated branding. |