

VARIANT*Plex*-HT Hereditary Cancer

Description

The VARIANT*Plex*-HT Hereditary Cancer 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*-HT standard protocol for Illumina® (RA-DOC-059) or VARIANT*Plex*-HT protocol for Illumina® (RA-DOC-058).

VARIANT*Plex*-HT Hereditary Cancer contains **2713** GSPs targeting 55 genes commonly mutated in hereditary cancers.

Description	Part number	Storage
VARIANT <i>Plex</i> -HT Hereditary Cancer GSP1 - 24 reactions	SA24463241	-20°C ± 10°C
VARIANT <i>Plex</i> -HT Hereditary Cancer GSP2 - 24 reactions	SA24463242	
VARIANT <i>Plex</i> -HT Hereditary Cancer GSP1 - 96 reactions	SA24463961	
VARIANT <i>Plex</i> -HT Hereditary Cancer GSP2 - 96 reactions	SA24463962	

Required reagent volumes

Protocol reference	Protocol step	Reagent	Volume per reaction (µL) per VARIANT <i>Plex</i> -HT standard protocol (RA-DOC-059)
A	Cleanup after Adapter Ligation	10mM Tris-HCl pH 8.0	20
B	First PCR	VARIANT <i>Plex</i> -HT Hereditary Cancer GSP1	8
C	First PCR	Purified DNA	18
D	Cleanup after First PCR	10mM Tris-HCl pH 8.0	16
E	Cleanup after First PCR	Purified DNA	14
F	Second PCR	VARIANT <i>Plex</i> -HT Hereditary Cancer GSP2	8

Protocol reference	Protocol step	Reagent	Volume per reaction (µL) per VARIANT <i>Plex</i> -HT protocol (RA-DOC-058)
A	Ligation Step 2 Elution	5mM NaOH	20
B	First PCR	VARIANT <i>Plex</i> -HT Hereditary Cancer 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 Hereditary Cancer GSP2	8

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	62	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

VARIANTPlex-HT Hereditary Cancer libraries should be sequenced to a minimum of 1.5M reads for germline applications and 8M reads for standard tumor profiling. 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 (v7.3, or greater). The VARIANTPlex-HT Hereditary Cancer panel is compatible with the **SNV/Indel**, **Structural Variations** and **CNV 2.0** pipelines, found under the **DNA** Input Type. Selection of the DNA Target Coverage pipeline is also optional and requires a region of interest BED file. See the Archer Analysis User Guide for more details on setting up your analysis.

Processing of VARIANTPlex-HT Hereditary Cancer libraries requires a one-time upload of a Target Region file (a text file, in GTF format, which directs the software on how to analyze data from the panel). For SNV/Indel detection it is recommended analysis is performed using a Targeted Mutations File. Files can be obtained by contacting archer-tech@idtdna.com

Assay Targets

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

<i>APC</i> ‡	<i>BRCA2</i> *	<i>DICER1</i>	<i>KIT</i>	<i>MSH3</i>	<i>PMS2</i>	<i>SDHA</i>	<i>STK11</i>
<i>ATM</i>	<i>BRIP1</i>	<i>EGFR</i>	<i>MAX</i>	<i>MSH6</i>	<i>POLD1</i>	<i>SDHAF2</i>	<i>TMEM127</i>
<i>AXIN2</i>	<i>CDH1</i>	<i>EPCAM</i>	<i>MBD4</i>	<i>MUTYH</i>	<i>POLE</i>	<i>SDHB</i>	<i>TP53</i>
<i>BAP1</i>	<i>CDK4</i>	<i>FH</i>	<i>MEN1</i>	<i>NF1</i>	<i>PTEN</i>	<i>SDHC</i>	<i>TSC1</i>
<i>BARD1</i>	<i>CDKN2A</i>	<i>FLCN</i>	<i>MET</i>	<i>NTHL1</i>	<i>RAD51C</i>	<i>SDHD</i>	<i>TSC2</i>
<i>BMPR1A</i>	<i>CHEK2</i>	<i>GREM1</i>	<i>MLH1</i>	<i>PALB2</i>	<i>RAD51D</i>	<i>SMAD4</i>	<i>VHL</i>
<i>BRCA1</i> *	<i>CTNNA1</i>	<i>HOXB13</i>	<i>MSH2</i>	<i>PDGFRA</i>	<i>RET</i>	<i>SMARCA4</i>	

†Contact adx-tech@idtdna.com for the panel target file to view complete list of targeted regions.

‡APC promoter 1A and 1B targeted for CNV

*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 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.

This product or service is licensed under one or more of the following U.S. Patents: 8,835,358; 9,290,808; 9,290,809; 9,315,857; 9,708,659; and 9,816,137 owned by BD and is licensed solely for the use described in the associated product literature. No other rights, implied or otherwise, are granted to purchaser hereunder. Purchaser agrees, by way of example and not limitation, not to use this product to trace back the origin of a nucleic acid to an individual cell as a discrete entity (e.g., single cell analysis).

© 2023 Integrated DNA Technologies, Inc. All rights reserved. FUSIONPlex, VARIANTPlex, LIQUIDPlex, IMMUNOVerse, Archer Analysis, and Archer Assay Marketplace are trademarks of Integrated DNA Technologies, Inc. All other marks are the property of their respective owners. For specific trademark and licensing information, see www.idtdna.com/trademarks.

Revision History

Document Number	Date	Description of change
RA-DOC-073/REV01	July 2024	Initial release.