

FUSION*Plex*-HT Lung v2

Description

The FUSION*Plex*-HT Lung v2 panel is a balanced pool of gene-specific primer (GSP) oligonucleotides that is optimized for use with FUSION*Plex*-HT reagents and molecular barcode (MBC) adapters to produce targeted NGS libraries. This product insert should be used in conjunction with FUSION*Plex*-HT protocol for Illumina® (RA-DOC-049).

FUSION*Plex*-HT Lung v2 contains **323** GSPs targeting **17** genes commonly mutated in non-small cell lung cancer (NSCLC).

Description	Part number	Storage
FUSION <i>Plex</i> Lung v2 GSP1, 24 reactions or FUSION <i>Plex</i> Lung v2 GSP1, 96 reactions	SA18090241 or SA18090961	-20°C ± 10°C
FUSION <i>Plex</i> Lung v2 GSP1, 24 reactions or FUSION <i>Plex</i> Lung v2 GSP1, 96 reactions	SA18090242 or SA18090962	
10X VCP Primer Mix	SA0126	

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

Recommended PCR cycling (cont.)

	Step	Temperature (°C)	Time	Cycles
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

FUSIONPlex-HT Lung v2 libraries should be sequenced to a minimum of **1M** 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, or greater). The FUSIONPlex-HT Lung v2 panel requires selection of the **Fusion** pipeline and (optional) **SNV/Indel** pipeline, found under the **RNA** Input Type. See the Archer Analysis User Guide for more details on setting up your analysis.

Processing of FUSIONPlex-HT Lung v2 libraries requires a one-time upload of the Custom Panel GTF. Files can be obtained by contacting archer-tech@idtdna.com.

Assay targets

Gene	Accession	Exon	Variant Type	Description**
ALK	NM_004304	2, 4, 6, 8, 10, 12, 14, 16, 17, 18, 19, intron19, 20, mid-exon20, 21, 22, 23, 26	Fusion, ALK ATIV, Internal deletion (ALKΔ2-17, ALKΔ2-3)	5'
ALK	NM_004304	1, 2	Internal deletion (ALKΔ2-17, ALKΔ2-3)	3'
ALK	NM_004304	22, 23, 25	Mutation	p.P1153-p.C1156, p.F1174, p.L1196-p.S1206, p.G1269

Gene	Accession	Exon	Variant Type	Description**
<i>BRAF</i>	NM_004333	2, 3, 4, 5, 7, 8, 9, 10, 11, 12, 15, 16	Fusion, Kinase Domain Duplication, BRAFΔ2-10, BRAFΔ4-10, BRAFΔ2-8, BRAFΔ3-8, BRAFΔ4-8	5'
<i>BRAF</i>	NM_004333	1, 2, 3, 7, 8, 10, 13, 14, 18	Fusion, Kinase Domain Duplication, BRAFΔ2-10, BRAFΔ4-10, BRAFΔ2-8, BRAFΔ3-8, BRAFΔ4-8	3'
<i>BRAF</i>	NM_004333	15	Mutation	p.V600
<i>EGFR</i>	NM_005228	7, 8, 9, 14, 15, 16, 17, 18, 19, 20	Fusion, Exon 2-7 Skipping (EGFRvIII), Kinase Domain Duplication	5'
<i>EGFR</i>	NM_005228	1, 24, 25, mid-exon25, 26	Fusion, Exon 2-7 Skipping (EGFRvIII), Kinase Domain Duplication	3'
<i>EGFR</i>	NM_005228	18, 19, 20, 21	Mutation	p.E709-p.G719, p.E746-p.L760, p.V774-p.G796, p.L858-p.L861
<i>ERBB2</i>	NM_004448	4, 5, 13, 15, 17	Fusion, Exon 16 skipping (Δ16HER)	5'
<i>ERBB2</i>	NM_004448	15, 23, 24, 25, mid-exon26, 26	Fusion, Exon 16 skipping (Δ16HER)	3'
<i>ERBB2</i>	NM_004448	8, 20	Mutation	p.G309-p.S310, p.Y772-p.P780, p.C805
<i>FGFR1</i>	NM_015850	2*, 3, 4, 5, 6, 7, 8, 9, 10, 11, 17	Fusion, Kinase Domain Duplication	5'
<i>FGFR1</i>	NM_015850	12, 17	Fusion, Kinase Domain Duplication	3'
<i>FGFR1</i>	NM_023110	4, 13, 14	Mutation	p.T141, p.V561, p.K656
<i>FGFR2</i>	NM_000141	2*, 3, 5, 6, 7, 8, 9, 10	Fusion	5'
<i>FGFR2</i>	NM_000141	16, 17, 18	Fusion	3'
<i>FGFR2</i>	NM_000141	7, 9, 12, 13, 14	Mutation	p.S252-p.P253, p.G305, p.Y375-V395, p.I547-p.N549, p.V564, p.A648-p.K659

Gene	Accession	Exon	Variant Type	Description**
<i>FGFR3</i>	NM_000142	3, 5, 8, 9, 10, 11, 12, 13, 14	Fusion	5'
<i>FGFR3</i>	NM_000142	16, 17, intron17, mid-exon18	Fusion	3'
<i>FGFR3</i>	NM_000142	7, 9, 13, 14, 16	Mutation	p.R248-p.S249, p.G370-p.R399, p.V555, p.D641-p.K650, p.G697-p.K715
<i>KRAS</i>	NM_004985	2, 3, 4	Mutation	p.G12-p.G13, p.Q61, p.K117, p.A146
<i>MET</i>	NM_000245	2, 4, 5, 6, 13, 14, 15, 16, 17, 21	Fusion, Exon 14 Skipping (METΔex14)	5'
<i>MET</i>	NM_000245	2, 13	Fusion, Exon 14 Skipping (METΔex14)	3'
<i>NRG1</i>	NM_001159996	1*, 3, 4, 5	Fusion	5'
<i>NRG1</i>	NM_004495	1, 2, 3, 4, 5, 6	Fusion	5'
<i>NRG1</i>	NM_013958	1*	Fusion	5'
<i>NRG1</i>	NM_013959	1*, 3	Fusion	5'
<i>NRG1</i>	NM_013962	1*	Fusion	5'
<i>NRG1</i>	NM_013962	1	Fusion	3'
<i>NTRK1</i>	NM_001007792	1, 2	Fusion	5'
<i>NTRK1</i>	NM_002529	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14	Fusion	5'
<i>NTRK1</i>	NM_002529	13, 14, 15, 16, 17	Mutation	Full kinase domain coverage for resistance mutations including p.G595
<i>NTRK2</i>	NM_006180	4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18	Fusion	5'
<i>NTRK2</i>	NM_006180	11, 14	Fusion	3'
<i>NTRK2</i>	NM_006180	16, 17, 18, 19, 20, 21	Mutation	Full kinase domain coverage for resistance mutations

Gene	Accession	Exon	Variant Type	Description**
<i>NTRK3</i>	NM_001007156	15	Fusion	5'
<i>NTRK3</i>	NM_002530	3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16	Fusion	5'
<i>NTRK3</i>	NM_002530	13, 14, 15, 17	Fusion	3'
<i>NTRK3</i>	NM_002530	15, 16, 17, 18, 19	Mutation	Full kinase domain coverage for resistance mutation detection including p.F617, p.G623, p.G696
<i>NUTM1</i>	NM_175741	2*, 3, mid-exon3, 4, 5, mid-exon6, 6	Fusion	5'
<i>PIK3CA</i>	NM_006218	2, 15	Fusion	5'
<i>PIK3CA</i>	NM_006218	2, 3, 5, 6, 8, 10, 14, 21	Mutation	p.E81K-p.G118D, p.L339-p.D350, p.G364R, p.E418-p.C420, p.E453-p.K468, p.P539-p.Q546, p.E726, p.Y1021-p.T1052
<i>RET</i>	NM_020630	2, 4, 6, 8, 9, 10, 11, mid-exon11, 12, 13, 14	Fusion	5'
<i>RET</i>	NM_020630	15, 16	Mutation	p.A883, p.M918
<i>ROS1</i>	NM_002944	2, 4, 7, 31, 32, 33, 34, 35, 36, 37	Fusion	5'
<i>ROS1</i>	NM_002944	38	Mutation	p.G2032

*Indicates exons that are entirely untranslated region (UTR), or for which the UTR is targeted.

**The mutations listed under the Description column are targeted by the assay design. Version 6.2 and earlier of Archer Analysis may not support RNA SNV/Indel variant calling at exon junctions depending on the sequence context (SNVs ≤5bp, indels ≤30bp). *De Novo* RNA SNV/Indel and Internal Tandem Duplication mutation detection are not supported on the Ion Torrent Sequencing System.

†ALK-ATI currently requires review outside of Archer Analysis.

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
RA-DOC-450/REV01	October 2023	Initial release.
RA-DOC-450/REV02	November 2023	Updated First and Second PCR cycling conditions to include separate anneal and extended steps. Updated branding.